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# **Use of near infrared spectroscopy to predict wood traits in *Eucalyptus* species**

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by

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## Summary

### Background

New Zealand is known for radiata pine (*Pinus radiata*) plantations, a pale coloured and non-durable timber. It is used in many agricultural and industrial sector products such as posts and poles. Wood preservatives are used to protect the non-durable timber against decay. One of the most widely used wood preservatives is the inorganic water-based preservative copper chrome arsenate (CCA). Wood preservatives give rise to environmental problems. CCA has been largely banned for most uses in the USA, Europe and Australia. There is a large demand for natural durable timbers in both the domestic market and international market. Naturally durable timbers often come from tropical forests and are frequently supplied from illegal/unsustainable sources. Such timber also has colour and is often used in the furniture industry. According to the NZ Ministry of Primary Industries' annual forestry imports statistics (<https://catalogue.data.govt.nz/dataset/annual-forestry-import-statistics>), over \$50 million of sawn hardwood timber and \$36 million of wooden furniture were imported by New Zealand in 2017 as a result of a lacking suitable domestic resource.

Some eucalyptus species have high natural durability and colour. The natural durability makes them ideal for wide-ranging agricultural and industrial use, particularly for posts and poles. *Eucalyptus bosistoana* has class 1 natural durability and a fast growth rate in New Zealand's climate. *E. bosistoana* can grow up to 30-40 m in height and usually has wood of excellent stiffness, high density and hardness.

The New Zealand Dryland Forests Initiative (NZDFI) was established in 2008 and aims to establish a sustainable naturally durable timber industry in New Zealand. It choose *E. bosistoana* as the main durable species for genetic improvement. NZDFI's durable eucalypts

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will produce high value naturally durable timber to meet the future demand of durable timber and replace CCA and illegal tropical hardwood.

A successful plantation industry needs to ensure quality (i.e. natural durability of the wood). Due to differences in genetics, environment and tree age, heartwood quantity and natural durability vary significantly within a specie and tree. Breeding can be an efficient way to reduce variability.

The primary objectives of this research were:

- 1) Develop a method to quickly assess heartwood traits for inclusion in a large scale breeding programme.
- 2) Assess genetic control over heartwood traits in *E. bosistoana*.

To achieve these objectives, this thesis also investigated:

- a) The distribution of extractives within a tree.
- b) Optimisation of the sampling technique.
- c) The possibility to apply the technique to other durable eucalypts species in the breeding programme.

## **Chapter 1: General Introduction and literature review**

A literature review highlighted that to obtain high quality for naturally durable wood, it is necessary and possible to ensure consistency through a breeding programme. Traditional methods to assess heartwood are time- and cost-consuming and need to be replaced to allow inclusion of heartwood traits in a tree breeding programme. Near infrared (NIR) spectroscopy has been successfully used to analyse heartwood.

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## **Chapter 2: Effects of variable selection and processing of NIR and ATR-IR spectra on prediction of extractives content in *Eucalyptus bosistoana* heartwood**

The use of quick attenuated total reflectance infrared (ATR-IR) spectroscopy and NIR spectroscopy to predict extractives content (EC) in the heartwood of *E. bosistoana* with partial least squares regression (PLSR) models was studied. Different spectra pre-processing methods and variable selection were tested for calibration optimisation. While variable selection substantially improved the NIR-PLSR models, only small effects were observed for spectra pre-processing methods and ATR-IR-PLSR models. Both, the NIR-PLSR and ATR-IR-PLSR models yielded reliably EC results with high  $R^2$  and low root mean square error (RMSE). NIR based models performed better (RMSE 0.9%) than ATR-IR based models (RMSE 1.6%). Analysis showed that the models were based on IR signals assigned to chemical structures known from eucalyptus heartwood extracts. Combined with PLSR and variable selection, both, ATR-IR and the NIR spectroscopy, can be used to quickly predict EC in *E. bosistoana*, a measure needed in tree breeding and quality control of for durable timber.

## **Chapter 3: Predicting extractives content of *Eucalyptus bosistoana* F. Muell. heartwood from stem cores by near infrared spectroscopy**

Time and resource are the restricting factors for the wider use of chemical information of wood in tree breeding programmes. NIR spectroscopy offers an advantage over wet-chemical analysis in these aspects, this work describes the development of a NIR-based assessment of EC in the heartwood of *E. bosistoana*, which does not require milling and conditioning of the samples. This was achieved by applying signal processing algorithms (external parameter orthogonalisation (EPO) and significance multivariate correlation (sMC)) to spectra obtained

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from solid wood cores, which were able to correct for moisture content, grain direction and sample form. The accuracy of EC predictions was further improved by variable selection, resulting in a root mean square error of 1.27%. Considering the range of EC in *E. bosistoana* heartwood of 1.3 to 15.0%, the developed NIR calibration has the potential to be used in an *E. bosistoana* breeding programme or to assess the special variation in EC throughout a stem.

#### **Chapter 4: Application of Near Infrared spectroscopy to heartwood of three durable eucalypts for quantification of extractives and species identification**

This chapter describes the use of NIR spectroscopy to quickly determine the EC in the natural durable heartwood of the three eucalypts, *Eucalyptus bosistoana*, *E. globoides* and *E. argophloia*. PLSR prediction models combined with variable selection considering all species predicted the EC with a residual mean square error (RMSE) of 0.90%, a better result than achieved for smaller datasets from single species. Considering the EC range of 0.34 to 18.85%, this method can be used to select durable breeds, an otherwise time and resource consuming property to measure. Key signals in the NIR spectra for the PLSR models were shared between the species and related to common chemical structures reported from eucalyptus heartwood extracts. Furthermore, it was possible to determine the species from NIR spectra with 100% accuracy, indicating that NIR spectroscopy can be used as a non-destructive method to segregate timber from mixed-species forest plantations.

#### **Chapter 5: Distribution of extractives within trees**

The radial and vertical of EC distributions in roots and tree stems were studied. In older trees, the inner heartwood showed a lower EC than the outer part of heartwood. Roots contained heartwood. The middle section of roots had the largest heartwood area and highest EC. In

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stems of older trees, EC was highest at medium height. Young tree stems and roots did not show a marked radial profile of EC in heartwood.

## **Chapter 6: Genetic variation in heartwood properties and growth traits of *Eucalyptus bosistoana***

Forty-one *E. bosistoana* families were evaluated for the production of heartwood quantity and quality in two sites. High estimated heritabilities of heartwood diameter (HWD) were found in both sites ( $h = 0.66$  and  $0.71$ ). The estimated heritabilities of EC were lower with  $0.16$  and  $0.25$ . Weak genetic correlations between HWD and EC were found in one site, but highly negative ( $-0.86$ ) genetic correlations were observed in the other. The  $G \times E$  interaction had no significant influence on growth traits but a small level influence on EC. Five families were selected for tree breeding as they produced both, large HWD and high EC, in both sites. The data suggested that genetic breeding selection could improve the heartwood quantity and quality of *E. bosistoana* plantations.

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## Chapter 1 **General Introduction**

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### **1.1 Introduction**

Wood is a bio-material and biodegradable. Being biodegradability is a positive attribute when considering the disposal at the end of a product's life. However, the susceptibility of wood to decay by organisms can result in premature product failure. Biodegradation of wood is particularly rapid in moist conditions with ground contact - e.g. for wooden poles. The environment in which wood is used is described by Hazard Classes (NZS 3640, 2003). The natural resistance of timbers against biodegradation is highly variable. While some timbers decay quickly, others can withstand moist in-ground conditions for a considerable time (Bootle, 2005; Scheffer and Morell, 1998). This property is referred to as (natural) durability and assessed by various national standards (ASTM, 2005; AWPC, 2007; EN 350-1, 1994). Unfortunately, high natural durability is uncommon among tree species and those which are utilised are mostly rare and/or harvested unsustainably (UNEP, 2012). As an alternative, the resistance to biological decay of non-durable timber can be improved by modifying the chemical structure of the wood (Hill, 2006) or by impregnation with one of the many available preservatives (Eaton and Hale, 1993; Goodell et al., 2003; Richardson, 1993). The former has only recently been used on an industrial scale and achieves either only lower durability (thermal modification) or is costly (acetylation). The latter has been used extensively for decades but converts the bio-degradable wood into a toxic waste for which

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often no acceptable disposal option exists (Graham, 2009; Read, 2003; Townsend and Solo-Gabriele, 2006).

Biodegradation of wood is caused by fungi, bacteria, insects and marine organisms.

- Fungal decay (Schmidt, 2006)

Numerous fungi are able to enzymatically decompose the chemical constituents (hemicelluloses, cellulose and lignin) from which wood is made. These fungi use the wood constituents as their energy source. Different types of fungal decay have been described (white-rot, brown-rot, or soft-rot). These differ in appearance and in the type of enzymes that decompose the wood. In all cases, the result is a complete loss of structural integrity, i.e. strength. Prerequisites for fungal decay are the presence of free water and oxygen. This implies that all wood in air-dry and anaerobic water-logged conditions is safe from fungal decay. Fungal decay is an important consideration when wood is used in ground contact, for example as posts and poles.

- Sap stain (Zabel and Morrell, 1992)

Some wood-colonising fungi do not have the enzymes required to break down the structural wood cell wall components (i.e. hemicelluloses, cellulose and lignin) but can feed on reserve materials (mostly starch), present in sapwood. Therefore, heartwood is not affected by sap stain. These fungi do not compromise the structural properties of wood but do pose a hygienic issue and discolour the wood.

- Bacteria (Clausen, 1996; Greaves, 1971)

Bacteria have also been reported to enzymatically break-down wood components. This can occur in oxygen deficient environments. However, the process is slow and does not pose a significant threat to timber.

- Wood-degrading beetles (Peters et al., 2002)

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Some beetles, like lyctids or anobiids (e.g. house borer) destroy wood mechanically by chewing. They are not able to feed directly on the cell wall components like wood-decaying fungi. They usually digest the tree's starch reserves which are present in sapwood only. Some (e.g. ambrosia beetles) rely on symbiotic fungi to break down wood components. As insects do not require the presence of free water sapwood can be prone to attack even when used inside buildings in air-dry conditions. Some ants and other insects can cause similar damage.

- Termites (Ahmed et al., 2004; Shelton and Grace, 2003)

Termites also destroy wood mechanically. They, however, can also use wood cell walls as an energy source with the help of symbiotic gut bacteria. Termites usually colonise wood in soil contact but some species can build tunnels to reach wooden structures above ground. Termites are most problematic in tropical/subtropical regions and termite-resistant woods are in high demand for construction in these parts of the world.

- Wood-degrading marine organisms (Cragg et al., 1999; Eaton et al., 1989; Nishimoto et al., 2015)

Some molluscs (e.g. Teredinidae - shipworms) and crustaceans (e.g. Limnoriidae - wood lice) use wood as habitat and food. These species need to be considered when wood is to be used in marine constructions like piers. Only a few timbers last a significant time in marine conditions.

The natural durability according to the Australian standard "Timber – Natural durability ratings" (AS5604, 2005) for NZDFI species is listed in Table 1-1. These ratings have been determined from timber harvested from old-growth natural forests in Australia, and timber of the same species from other sources, especially short-rotation plantations, can compare unfavourably.



**Table 1-1 Natural durability ratings of NZDFI species according to (AS5604, 2005) and heartwood colour (Bootle, 2005). <sup>a</sup> See (Cookson, 2004), <sup>b</sup> See (Cookson et al., 2009), <sup>c</sup> See (AWPC, 2007).**

Species	Lyctid susceptibility of sapwood	Termite resistance of heartwood	In-ground life expectancy (years)	Above- ground life expectancy (years)	Life expectancy in southern waters (years)	Colour
<i>Eucalyptus bosistoana</i>	Susceptible	Resistant	>25 <sup>a</sup>	>40	21 to 40	Pinkish pale brown
<i>Eucalyptus argophloia</i>	Susceptible <sup>b</sup>	ND	ND	ND	ND	Orange- brown to deep red- brown <sup>c</sup>
<i>Eucalyptus quadrangulata</i>	Not susceptible	Resistant	15 to 25	15 to 40	ND	Pale yellow
<i>Eucalyptus sideroxylon</i>	Susceptible	Resistant	>25	>40	41 to 60	Dark red
<i>Eucalyptus globoidea</i>	Not susceptible	ND	15 to 25	ND	21 to 40	Pinkish pale brown

### 1.1.1 Basis for natural durability

The large differences in resistance to fungal decay between timber species are predominately caused by its chemical composition, i.e. the secondary metabolites deposited in heartwood (Hawley et al., 1924; Rudman, 1963; Schultz et al., 1995). The effect of wood density on fungal decay is less clear as higher density wood often coincides with a higher concentration of secondary metabolites, known as the ‘extractive content’. It has been reported that density has a positive effect on decay resistance (Bush, 2011; Cookson and McCarthy, 2013; Edlund,

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1998; Plaschkies et al., 2014; Sehlstedt-Persson and Karlsson, 2010; Wong et al., 1983; Yu et al., 2003). Increased density has been shown to significantly reduce mechanical damage by insects and marine organisms (Peters et al., 2014). However, these results were based on comparisons between timber species and such large variation is not necessarily present within a species (Cragg et al., 2007). Other factors contribute to the natural resistance of wood to biological decay, for example, pore size (Peters et al., 2002) or permeability (Bush, 2011).

In most trees the wood in a stem changes from sapwood to heartwood some years after formation (Beakbane et al., 1971; Hillis, 1987; Rowe, 1989; Taylor et al., 2002). The first year rings, closest to the bark, are young and contain some living cells, called parenchyma. This wood is defined as sapwood and contains reserve materials like starch. After a species-specific period of years the reserve materials are removed from the older parenchyma and the parenchyma cells die. Following this transition the tissue is called heartwood and it starts forming at the centre of the stem, while new sapwood continues to be formed closest to the bark. During heartwood formation, some species synthesise numerous smaller organic compounds, which are deposited into the wood. These compounds are called heartwood extractives. Heartwood extractives are not a structural part of the wood cell wall, but some of the compounds are coloured or have bioactive properties, which can result in an attractive timber colour and high resistance against biodegradation. Sapwood is never considered as naturally durable (AS5604, 2005) nor is it coloured other than the typical pale brown (Kohl, 2012).

Another important change in the wood properties with time is a decrease in permeability. To reduce the risk of embolism, the transport function of the tissue is removed by pit aspiration, tylosis or deposition of extractives. This can precede heartwood formation (Ziegler, 1968) and is thought to reduce the susceptibility for wood to biodegradation.

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### 1.1.2 Variability and control of heartwood traits

Within a species variability in wood properties has economic consequences as timber products and wood processing benefit from consistent and adequate properties (Walker, 2006). Some of the variation in wood properties is under genetic control, enabling the selection of superior trees, i.e. healthy trees which produce larger quantities of good quality timber. Like other factors, heartwood properties vary between individuals, but variation of heartwood extractives and natural durability within a stem has also been reported. Within-stem variation exists as characteristic spatial pattern as well as random local variations. Environmental parameters have been found to influence this variation, highlighting the need to address heartwood in site-species matching and growth and yield modelling (Sharma et al., 2014).

#### 1.1.2.1 Within tree variability of heartwood features

The natural durability of heartwood decreases towards the pith (Sherrard and Kurth, 1933; Taylor et al., 2002). This is recognised in relevant standards, for example it is stated in AS5604 (2005) that:

*“... the inner heartwood (the first few growth rings around the pith), generally, has a lower natural durability than the rest of the heartwood.”*

The typical radial pattern of heartwood durability is mirrored by the amount of extractives in the stem (Sherrard and Kurth, 1933; Taylor et al., 2002). Additionally slight increases in heartwood durability were reported with stem height. This is in analogy to wood ‘quality’ of juvenile pine corewood (Burdon et al., 2004), which generally has unfavourable properties for most applications.

Heartwood extractives can also vary considerably in their abundance on a micro scale. For a durable product a homogeneous distribution within cell walls, between cell types (e.g. fibres,

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parenchyma) and within year rings is desirable (Taylor et al., 2002). Conversely a variable EC can be the defining feature in the appearance of timbers like *Microberlinia brazzavillensis* (Kohl, 2012) or *Dacrydium cupressinum*.

#### **1.1.2.2 Genetic control of heartwood features**

Previous studies have reported on the variability and the degree of genetic control of heartwood features in various tree species e.g. (Bush et al., 2011; Denis et al., 2013; Dos Santos et al., 2004; Miranda et al., 2014; Poke et al., 2006; Stackpole et al., 2010). The research on within tree variation is limited, however, substantial within species variation in durability has been demonstrated. Although the durability of heartwood from young trees is generally lower than that of old trees, individuals having class 1 durable heartwood at young age have been reported (Bush, 2011; Palanti et al., 2010). Therefore the production of ground-durable posts from young, short-rotation plantations should be possible by selecting a genetically superior resource.

A wide range of ECs have been observed in heartwood samples of *E. bosistoana*. Ethanol soluble extractives varied between 1.5% to 15% (wt/wt basis) at age 4½ (McLaughlin, 2013; Sharma et al., 2014), indicating that trees of good durability at a young age can be found.

A recent study on the variability in heartwood traits of hybrid larches reported heritabilities of >0.65 for heartwood diameter and EC (total phenolics) (Paques and Charpentier, 2015). Genetic gains of 10% in heartwood diameter and phenolics content were achievable. Furthermore, it was reported that the influence of female (European larch) and male (Japanese larch) parents differed between the traits.

A good prospect for genetic improvement of *E. cladocalyx* heartwood quantity (%), methanol EC and durability was reported (Bush et al., 2011). The data did not indicate unfavourable

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genetic correlations between growth and durability. Similar results were found for other species.

Another heartwood feature determining the value of the timber is its colour. The genetic control of heartwood colour has been studied for several species (Gierlinger et al., 2004; Mosedale et al., 1996; Moya et al., 2013; Rink and McBride, 1993).

### 1.1.3 Chemistry of heartwood extractives

Little is known about the identity of the chemical compounds in heartwood of eucalypts included in NZDFI's breeding programme (*E. bosistoana*, *E. argophloia*, *E. tricarpa*, *E. quadrangulata* and *E. globoidea*). Research on the chemical heartwood compounds of other durable eucalypts has been conducted in the past (Hillis, 1991). In recent times the research focus has been on non-durable eucalypts species grown for pulp and paper, for which low EC are desirable (Swan and Akerblom, 1967). Chemotaxonomy of eucalypts by heartwood (Hathway, 1962) and leaf (Hillis, 1966; Hillis and Inoue, 1967; Padovan and Manzan, 2014) compounds has been explored and bark and root extractives of eucalypts have also been studied (Cadahia et al., 1997a, b; da Seca and Domingues, 2006; Dayal, 1987).

The heartwood compounds within a species are numerous (Hillis, 1987; Rowe and Conner, 1979), with each molecule having different properties influencing, for example bioactivity (Morris and Stirling, 2012; Ohtani et al., 2009), colour (Balogh and Anderson, 1965; Takahashi and Mori, 2006; Zavarin and Smith, 1962) or interaction with glues (Wang, 1992). Furthermore the relative proportions of the individual compounds vary within species (Daniels and Russell, 2007; Niamke et al., 2014). Therefore it is important to understand how both the absolute amount of extractives and the extractive composition affect wood properties like durability and colour. As the biosynthesis of the individual compounds is also partly under genetic control (Fries et al., 2000) the possibility exists to further improve wood quality

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by breeding. A requirement is the identification of the key compounds in the heartwood for the NZDFI species.

Individual extractives from heartwood or other tree tissues can also be of value themselves. Numerous reports are published on natural compounds to be used as natural wood preservatives (Singh and Singh, 2012), in medicine or for other applications. For example a compound isolated from *E. globoidea* buds (Globoidnan A) has been found to be an inhibitor of HIV integrase (Ovenden et al., 2004). Recently the variability in the essential oils extracted from *E. bosistoana* leaves has been reported (Bouzabata et al., 2014). Tannins from eucalypts are other compounds of interest (Cadahia et al., 1997b).

#### 1.1.4 Measuring heartwood volume

The relative amounts of sapwood and heartwood in a tree can have a marked impact on its value. For naturally durable NZDFI species, high heartwood content is desirable. However, sapwood is desired for pulp production and other wood manufacturing processes as heartwood can drastically increase processing costs (Morais and Pereira, 2007). Sapwood width is also a key factor for understanding water balances of forest plantations (Kumagai et al., 2005), which are increasingly in the spotlight with climate change and intensified agriculture. Therefore measuring the sapwood width in standing trees is not only of interest for research purposes but also for quality control in production forestry in a variety of settings. Unfortunately assessing sapwood depth in standing trees is not trivial.

It is possible to detect heartwood and sapwood on extracted cores, but this can be difficult for species, which do not have marked colour or moisture content differences between sapwood and corewood. For these cases colour stains may be available to highlight heartwood or sapwood (Hillis, 1987). Alternatively microscopy, X-ray tomography or NIR have been used to detect the heartwood sapwood boundary in cores (Pfautsch et al., 2012).

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Non-destructive methods have been developed in recent years. A transportable magnetic resonance imaging system has been developed, which can measure sap flow in standing trees (Jones et al., 2012), but in its current form the equipment size and weight as well as the limitations to stem diameter (~10 cm) prevent this tool from being used more widely.

Electrical resistivity tomography has also been used to detect sapwood in standing trees (Wang et al., 2016), but the reported accuracy of the method has been questioned for eucalypts (Pfautsch et al., 2016) and the need for multiple sensors limits its use for fast assessment of many trees for quality control of plantations or breeding trials.

#### **1.1.5 Measuring durability**

The standard methodology to assess the durability of timber is to measure the mass loss of a sample after exposure to wood degrading organisms either in field or under laboratory conditions. The details differ significantly depending on the hazard class tested for (AWPC, 2007). These tests require numerous (>6) reasonable sized (>20 mm) samples and may run for a considerable time (>12 weeks) (AWPC, 2007). The effort of measuring natural durability by mass loss has been recognised as prohibitive in large scale screening programmes (Bush et al., 2011; Paques and Charpentier, 2015).

Experimental alternatives to accelerate durability assessments have been proposed. The required exposure time can be shortened by creating more favourable environmental conditions that accelerate the decay (Cookson and McCarthy, 2013). Furthermore it is possible to use more sensitive techniques to assess decay in the early stages, before significant mass loss occurs. In the early stage of decay remarkable changes in the physical properties and the chemical composition of the wood can be observed. Acoustics, which are associated with the stiffness of a material, have been found useful to detect decay early (Machek et al., 2001). Several studies report that NIR can be used to predict the severity of

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decay in wood, based on changes in the chemical composition of the woody cell walls, removing the need to measure mass loss (Fackler and Schwanninger, 2012). However, these methods are still difficult to realise in a sizable breeding programme due to their resource demands.

As outlined above (1.1.1) the durability of wood is related to the EC in heartwood. Again, the resources needed for the conventional test method to determine the EC by solvent extraction of milled wood (TAPPI, 2007) are prohibitive for use in a screening programme (Takashima et al., 2015). Harju and Venäläinen (2006) proposed to use a more efficient Folin-Ciocalteu assay for total phenolics to more rapidly assess durability in *Pinus sylvestris*. Alternatively, NIR is a technique able to obtain information on the chemical composition of a material (Osborne et al., 1993) and is used for this purpose for agricultural products. Several studies have found NIR is able to accurately assess the EC of heartwood e.g. (Alves et al., 2012; Bush et al., 2011; Giordanengo et al., 2008; Poke et al., 2004; Stackpole et al., 2011). However, the prediction of mass loss by decay fungi was not always reported to be accurate enough to support a breeding programme e.g. (Bush et al., 2011; Gierlinger et al., 2003).

Wounding was suggested as an early testing method to breed for heartwood (Harju et al., 2009). The wound reaction in stems after injury is not identical but somewhat related to heartwood formation (Blanchette et al., 1992).

#### **1.1.6 NIR on wood and heartwood**

NIR spectroscopy is a promising method that has been used for the non-destructive rapid analysis of the chemical composition of biomaterials (Bailleres et al., 2002; Cen and He, 2007; Nicolai et al., 2007; Prieto et al., 2009; Roggo et al., 2007; Yu et al., 2009). NIR is a type of vibrational spectroscopy which refers to the spectral region from 750 to 2,500 nm (wavenumbers: 13,300 to 4,000  $\text{cm}^{-1}$ ) which mainly interacts with C-H, N-H, S-H, or O-H



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bonds (Pasquini, 2003). NIR spectra usually contain not only chemical information but also physical information from scattering. It has been used for qualitative and quantitative determination of different wood properties. Typically calibrations are built from a calibration set and a validation set. The calibration set is used for establishing the regression equations between NIR spectra and the given trait. The validation set is used for checking the accuracy of the regression equations (Jones et al., 2008).

NIR spectroscopy is being increasingly used in forestry and forest ecology (Tsuchikawa, 2007; Tsuchikawa and Schwanninger, 2013). Numerous studies are using NIR to rapidly measure wood traits. For example, Flæte and Haartveit (2004) used NIR as a tool to predict the decay resistance of *Pinus sylvestris* heartwood. Good correlations ( $r > 0.86$ ) with predicted mass loss were found. Taylor et al. (2011) estimated the individual extractives components of American white oak (*Quercus alba*) by NIR. Esteves and Pereira (2008) demonstrated potential to predict wood properties (mass loss, equilibrium moisture content, dimensional stability, MOE, bending strength, colour parameters and EC) in *P. pinaster* and *E. globulus* by NIR spectroscopy. Gierlinger et al. (2003), using PLSR of NIR data and sets of wood decay tests, showed that decay tests could be predicted by NIR in larch with high coefficients of correlation ( $R^2=0.92$ ) and low root mean square errors of prediction ( $RMSEP = 0.08$ ).

#### 1.1.7 Grain of wood

The orientation of cells in wood does not always align with the stem axis (Harris, 1989). The grain angle is defined as the angle between the cell and the stem axes. In spiral-grained trees, the grain angle is either left or right handed, while in trees with interlocked grain the grain angle switches regularly in radial direction between left and right handedness. Spiral grain has a negative effect on the mechanical properties of wood (Jozsef, 1982) and also leads to twist in dried sawn timber due to anisotropic shrinkage. It is considered as a wood defect for

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sawn timber and therefore determines the end use of wood products (Raymond, 2002). However, interlocked grain, which results in notable figure of wood without negative impact on mechanical wood properties is a desirable wood trait (Harris, 1989; Thinley et al., 2005). Measuring the grain angle is difficult relying on visual scribe tests, X-ray or microwaves (McLauchlan et al., 1973; Sepúlveda, 2001).

It has been reported that the grain angle of solid wood is affecting NIR spectra (Tsuchikawa et al., 1998). Schimleck et al. (2003) found that NIR spectra collected from the radial longitudinal face of air-dry *P. taeda* wood gave better calibration models than those from the transverse face. Gindl and Teischinger (2007) reported NIR could be a non-destructive method to determinate grain angle with a root mean square error of 6 °.

*E. bosistoana* wood has both spiral grain and interlocked grain (Bootle, 2005). Trees that express regular interlocked grain are of most value and could be a target trait for a breeding programme. If NIR has the potential to quickly predict the grain angle in wood, it would be possible to select for interlocked grain to produce high-quality appearance timber.

Another consideration regarding the above-mentioned effect of grain on NIR spectra of solid wood is the accuracy of prediction models for wood properties. In this thesis, heartwood quality is envisaged to be assessed by predicting EC from NIR spectra of stem cores. As the grain orientation is not always clearly identified on a cylindrical core and additionally can vary due to the inherent grain pattern of a tree (Harris, 1989), grain must be considered when calibrating NIR for assessment of wood properties from solid samples.

#### **1.1.8 Effects on NIR spectra**

Moisture content (MC) (Giordanengo et al., 2008) and samples preparation (Hein et al., 2010) may influence the accuracy of PLSR models to predict wood traits. NIR spectra are sensitive to the O-H vibrations which associated with water (Leblon et al., 2013). Giordanengo et al.

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(2008) found that an increase in MC significantly reduced the accuracy of NIR calibration models for phenol content in *Eucalyptus urophylla* × *E. grandis* hybrid wood. Fresh/green wood samples have high and variable MCs making NIR calibrations for traits other than MC challenging. Consequently wood is usually dried before NIR spectroscopy. However, dry wood is a hygroscopic material changing its MC which changes in relative humidity and temperature. Therefore good control over the climatic conditions are necessary to achieve good NIR results when working with dry wood.

NIR spectra are also influenced by the sample preparation, such as particle size, surface roughness and thickness (Faix and Böttcher, 1992; Hein et al., 2010). Hein et al. (2010) reported that solid wood surface roughness and particle size influenced NIR model prediction accuracy of lignin content in eucalyptus. Differences between solid and milled wood were larger than between the size of the powder particles and that the accuracy of milled samples was superior to solid wood samples. However, grinding the samples for NIR is time-consuming and destructive.

#### **1.1.9 NZDFI's approach for assessing heartwood in breeding populations**

NZDFI has established numerous breeding trials since 2009. The resources necessary to test natural durability in a breeding programme are prohibitive. Additionally, sample size (one 14 mm diameter increment core of each individual) is limited. Therefore, a potentially more durable resource is selected through the proxy of high heartwood EC. The trees with high EC are more likely to produce more naturally durable timber. As the trees age and start to form, heartwood families will be assessed on a) the amount of heartwood in the stem and b) the EC in the heartwood. Having characterised the genetic resource, it will be possible to select trees with abundant heartwood rich in extractives for propagation.

The actual natural durability rating of this selected resource needs to be determined according to standards by independent organisations at a later stage when trees reach harvest age. As

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this will only be done for the selected trees the resources required are reduced significantly compared to assessing the entire breeding population. Standards also vary depending on the envisaged export market.

For the breeding population the EC in the heartwood will be assessed by NIR spectroscopy on cores with a fibre optics probe as milling and subsequent extraction is resource demanding. In order to assess the EC in heartwood in this way the system needs to be calibrated for each NZDFI species.

#### **1.1.10 Tree corer**

The assessment of heartwood requires a heartwood sample. As trees in the breeding population cannot be felled, it is necessary to non-destructively extract a wood core for analysis. This is not a trivial task. The NZDFI species are of very high density making the use of the conventional hand corers impossible especially as we require larger diameter cores for the further measurements. CSIRO in Australia had designed a tree corer in the 1990's (Downes et al., 1997) to extract 12 mm diameter cores from eucalypts leaving a 22 mm hole. But its manufacturing had stopped in the 2000's and no tree corer was available internationally when NZDFI embarked on its wood quality programme. Only recently a version of TRECOR was made available again by Forest Quality (Australia). As TRECOR is driven by a petrol engine, its design is heavy and raised concern regarding fire safety in dry forests. Therefore a new tree corer was designed.

In collaboration with Callaghan Innovation (New Zealand), a battery-powered, light-weight tree corer was developed, which allows the easy and quick extraction of 14 mm diameter cores from a 21 mm hole, ideal for younger smaller diameter trees (Figure 1-1). Extraction of cores is quick, taking only ~30 s for a 15 cm diameter tree. A battery back lasts for 20-40

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cores depending on tree diameter. A team of 2 was able to core >1000 trees in a week including the assessment of heartwood quantity.



**Figure 1-1** Extracting a core from an *E. bosistoana* tree using a light-weight, battery-powered tree corer developed by the New Zealand School of Forestry.

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## Chapter 2 **Effects of variable selection and processing of NIR and ATR-IR spectra on prediction of extractives content in *Eucalyptus bosistoana* heartwood**

This chapter has been submitted as:

Li, Y., Altaner, C., Effects of variable selection and processing of NIR and ATR-IR spectra on prediction of extractives content in *Eucalyptus bosistoana* heartwood.

The manuscript presented here may differ from any published version due to peer-review and editorial processes.

### **2.1 Introduction**

Wood is a renewable natural material and used for many purposes ranging from general construction work to reconstituted products. However, like for all natural materials, its properties are highly variable even within a species. A successful timber industry relies on good and uniform wood quality. This can be achieved by segregation in tree breeding programs. Both approaches require rapid and cost effective methods to measure large numbers of samples. This is not trivial for many wood properties, but near infrared (NIR) and infrared (IR) spectroscopy have been shown potential as rapid, low-cost, non-destructive measurement techniques of various wood properties (Moore and Owen, 2001; Tsuchikawa, 2007; Tsuchikawa and Schwanninger, 2013). NIR spectra are typically acquired in reflection, reducing the demand on sample preparation. Reflection spectra can now also be obtained from powders without sample preparation in the IR range with attenuated total reflectance (ATR) IR spectroscopy.

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Multivariate data analysis, especially partial least square regression (PLSR), is typically used to obtain quantitative information from NIR and IR spectra (Bjorsvik and Martens, 2001). This calibration process is dependent on spectra pre-processing (Rinnan et al., 2009) and variable selection (Mehmood et al., 2012). Spectra modifications and variable selection attempt to minimise the effects of noise, baseline variation and additive effects arising from interfering physical and chemical factors (Xu et al., 2008). To obtain a reliable calibration model, the samples should be split in calibration and validation data sets. This is often done by Kennard-Stone sampling, which samples based on Euclidian distances (Kennard and Stone, 1969), for spectroscopy data as random selection can results in less accurate models (Zhang et al., 2017). In addition, IR spectra contain not only informative but also uninformative variables. Those uninformative variables can influence the robustness of the calibration models. Variable selection can improve the performance of calibration models by only selecting those wavelengths, which explain variation in the target variable (Mehmood et al., 2012). Of the many methods which have been developed for variable selection, filter methods can be easily combined with PLSR calibration models because variable selection is performed independently on model fitting (Mehmood et al., 2012). The significance multivariate correlation (sMC) filter method not only reduces the effect of unrelated variables but concurrently aids spectra interpretation as it highlights the variables that are most correlated to the response (Tran et al., 2014).

This study focuses on the quantification of the extractive content (EC) in heartwood of *Eucalyptus bosistoana*, which is fast-growing in New Zealand's climate and produces durable timber (class 1) (Bootle, 2005). *E. bosistoana* have been selected by the New Zealand Dryland Forests Initiative (NZDFI), which aims to establish a sustainable supply of ground durable timber for local and international markets (Altaner et al., 2017; Walker, 2011). The EC is the key factor for the natural durability of wood (Hawley et al., 1924; Rudman, 1964),

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which is very time consuming to measure directly (ASTM, 2005; Harju and Venäläinen, 2006). Therefore rapid assessment of EC can be a quick indirect assessment of durability allowing quantification of heartwood quality for genetic selection in tree breeding, which benefits from large numbers of individual tree samples. Both, NIR (Geladi et al., 2014; Maniwaru et al., 2014; Ribeiro da Silva et al., 2013) and IR (Meder et al., 1999) were reported suitable for assessing EC in wood. Predictions of mass loss caused by fungi based by IR spectroscopy was not always accurate (Bush et al., 2011; Jones et al., 2011). Depending on the application NIR or IR spectroscopy have been reported to be more accurate. For example, while IR spectroscopy outperformed NIR for the determination of  $\beta$ -carotene content in tomato fruits (Baranska et al., 2006), the opposite was reported for chemical features of hardwoods (Zhou et al., 2015).

The objectives of this work were 1) to investigate the potential of NIR and ATR-IR spectra to quantify the EC in *E. bosistoana* heartwood, 2) optimise the PLSR models based on for NIR and ATR-IR spectra and 3) obtain information on the chemical structure of heartwood compounds in *E. bosistoana*.

## **2.2 Methods and Materials**

### **2.2.1 Materials**

*E. bosistoana* trees were planted in 2009 by the New Zealand Dryland Forests Initiative (NZDFI) in Marlborough New Zealand. Discs with a length of ~10 cm were cut in May 2016 from the bottom of the tree trunk in two different sites (41°26'S, 173°56'E and 41°43'S, 174°02'E). In total, 100 *E. bosistoana* wood disc samples were collected. Each disc was air-dried (25 °C, 60% RH) for one month. The heartwood was isolated from all wood discs by drilling into the transverse face with a drill (12-mm). Sapwood from 10 randomly selected discs was collected in the same way. The drill 'dust' was ground in a Wiley mill fitted with a

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20 mesh (0.85 mm) screen. The powdered samples were then oven dried at 60 °C to a stable moisture content (MC) of ~2%.

### 2.2.2 NIR and ATR-IR spectroscopy

NIR and IR spectra were collected with a Bruker Tensor 37 spectrometer controlled with OPUS\_7.5.18 software (Bruker Optik GmbH, Germany). NIR spectra of wood powder were taken with a fibre-optics probe (Model N-500, Bruker Optik GmbH, Germany) at wavelength from 9000 to 4000  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  intervals averaging 32 scans. IR spectra were obtained with an ATR accessory in combination with a RT-DLaTGS detector (Bruker Optik GmbH, Germany) in the range from 4000 to 800  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  intervals averaging 32 scans. Three spectra were taken from each wood powder in both regions, and the corresponding spectra were averaged.

### 2.2.3 Extractive content

Approximately 5-8 g of oven dry wood powder was precisely weighted into a stainless-steel cell and extracted with ethanol in an Accelerated Solvent Extractor (ASE) (Thermo Scientific) using the following extraction conditions: static time of 15 mins, temperature of 70 °C, 100% rinse volume and 2 extraction cycles.

Dry aluminium foil trays of known masses were used to hold the extractive solutions and left in a fume hood overnight for the ethanol to evaporate. The extracts were subsequently oven dried at 105 °C. The dry mass of each extract was measured, and the EC was calculated on a dry mass basis.

NIR and ATR-IR spectra were collected of dry ethanol heartwood and sapwood extracts from ten selected trees and averaged.

### 2.2.4 Data processing



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The Kennard-Stone sampling method was used to divide the data into two subsets: 80 samples were selected for a calibration data set, and the remaining 20 samples were used as validation data set. R software (version 3.1.2) (R Core Team, 2017) was used for data processing. The prospectr package (Stevens and Ramirez–Lopez, 2014) was used for NIR spectra manipulation and Kennard-Stone sampling. Three spectral pre-processing methods were tested: standard normal variate (SNV), 1<sup>st</sup> derivative and 2<sup>nd</sup> derivative. The Savitzky-Golay algorithm with a second-order polynomial and a window size of 15 was used to calculate the 1<sup>st</sup> and 2<sup>nd</sup> derivatives. The pls package (Mevik et al., 2015) was used for developing the PLSR calibration models and optimal components selection with leave-one-out cross-validation. The sMC (alpha = 0.05) algorithm implemented by the plsVarSel package (Mehmood et al., 2012) was applied to both the NIR and ATR-IR spectra to a) study the effect of spectra pre-processing on the most important variables for PLSR models for heartwood EC and b) variable selection.

## 2.3 Results and discussion

### 2.3.1 Preliminary data analysis

The EC ranged between 0.96% and 14.67% in the calibration data set with an average of 5.64% (Table 2-1). The range was with 2.08% to 7.19% smaller for the validation data set.

**Table 2-1 Summary statistics of ethanol soluble extractive content (EC) in heartwood of 7 year-old *E. bosistoana* for the used datasets; CV: Coefficient of variation; n: number of selected samples.**

		Calibration data	Validation data
		(n = 80)	(n = 20)
EC	Max (%)	14.67	7.19
	Mean (%)	5.64	4.53
	Min (%)	0.96	2.08
	CV	0.61	0.41

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NIR and ATR-IR spectra of *E. bosistoana* are dominated by cell walls and extractives are only a minor component. To identify characteristic signals for heartwood extractives the averaged NIR and ATR-IR spectra of wood powders were compared to those of dry extractives (Figure 2-1). To resolve some signal overlap, the NIR spectra were converted into their 2<sup>nd</sup> derivatives (Schwanninger et al., 2011). The heartwood extractives showed unique signals at ~6900, 6017 and 4659 cm<sup>-1</sup> in the 2<sup>nd</sup> derivative NIR spectra. These were close to signals assigned to the 1<sup>st</sup> overtones of stretching vibrations of phenolic O-H (Fackler and Schwanninger, 2010) and C<sub>ar</sub>-H groups as well as combinations of vibrations C<sub>ar</sub>-H and C=O groups related to extractives in *E. globulus* (Michell and Schimleck, 1996), respectively. In the ATR-IR spectra, the heartwood extractives showed characteristic signals at 1712, 1330-1300, ~1174 and 860 cm<sup>-1</sup>. Signals ~1712 cm<sup>-1</sup> were assigned to stretching vibrations of C=O groups of ketones, esters and conjugated aldehydes (Faix, 1991), while a signal at ~1330-1300 cm<sup>-1</sup> was likely to be related to phenolic O-H groups (Schwanninger et al., 2004). Two broader peaks at around 1350 cm<sup>-1</sup> and between 1290 and 1150 cm<sup>-1</sup> for were reported to arise from combinations of C-O stretching and O-H deformation vibrations of hydrolysable and condensed tannins, respectively (Edelmann and Lendl, 2002). Vibrations from C-H groups were reported at 860 cm<sup>-1</sup> (Schwanninger et al., 2004). These assignments fit well to what is known on the chemical components in heartwood extracts of eucalypts, which were generally termed as polyphenol compounds including hydrolysable and condensed tannins (Hillis, 1972; Rudman, 1964). Sapwood of *E. nitens* was shown to contain only small amounts of phenolic compounds (Barry et al., 2000; Eyles et al., 2003).

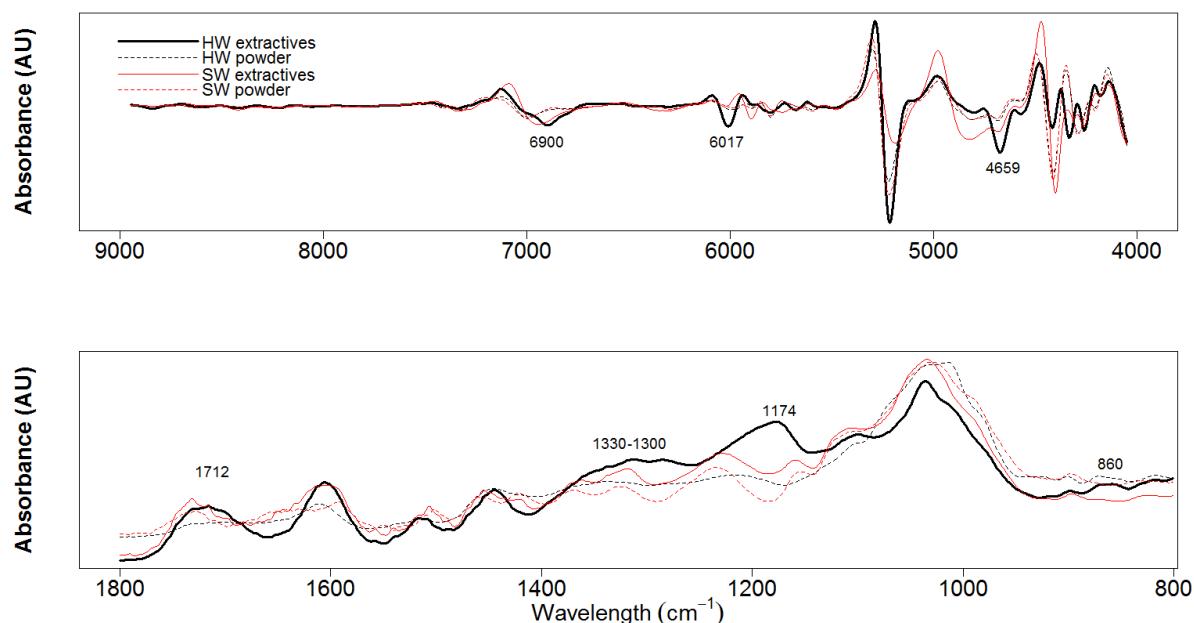


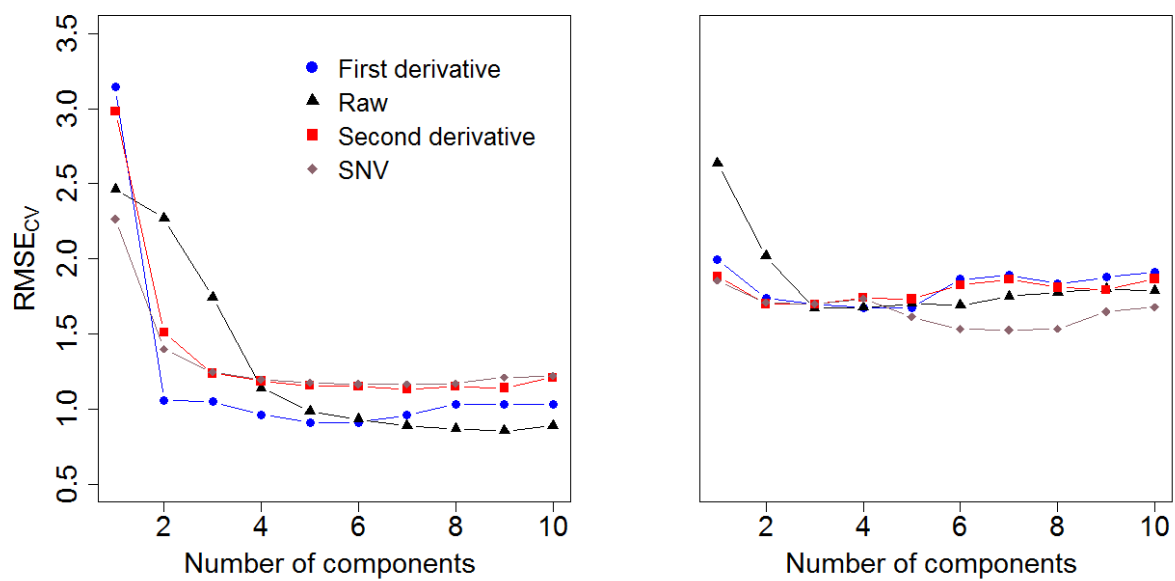
Figure 2-1 2<sup>nd</sup> derivate NIR (top) and ATR-IR (bottom) spectra of heartwood (HW) and sapwood (SW) extracts as well as wood powders of *E. bosistoana*; AU: arbitrary units.

As the characteristic heartwood signals overlapped with peaks from the cell wall polymers it was difficult to quantify the extractive content directly from the spectra. PLSR methods are typically used for quantitative analysis of IR spectra (Bjorsvik and Martens, 2001).

### 2.3.2 Effects of spectra pre-processing on PLSR models

The spectra can be negatively influenced by noise and baseline shifts. Spectral pre-processing methods are often used to reduce these effects on PLSR models (Rinnan et al., 2009). Four pre-processing methods were applied to both NIR and ATR-IR spectra of *E. bosistoana* wood powder and their effect on PLSR models for EC were assessed (Figure 2-2). Regardless of the pre-processing method, the PLSR models based on the NIR spectra performed better, with the residual mean square error (RMSE<sub>CV</sub>) ranging between 0.99 to 1.25%, than those based on ATR-IR NIR spectra (RMSE<sub>CV</sub> 1.67 to 1.74%). This effect was consistent with earlier reports showing that NIR spectra allowed more accurate prediction of wood properties than ATR-IR (Sun et al., 2011; Via, 2010; Zhou et al., 2015). SNV and 2<sup>nd</sup> derivative

transformations did not improve the model accuracy. For NIR spectra the models based on raw spectra and their 1<sup>st</sup> derivative performed best, however more components were needed using the raw spectra (Table 2-2). The applied spectra pre-processing methods had no effect on the performance of the ATR-IR models. The accuracy of the models were comparable to those reported for quantification of several chemical wood features by NIR and ATR-IR spectra (Zhou et al., 2015).



**Figure 2-2** Residual mean square error of calibration using leave-one-out cross validation ( $RMSE_{CV}$ ) for PLSR models predicting EC in *E. bosistoana* heartwood for of different spectra pre-processing of NIR spectra (left) and ATR-IR spectra (right). SNV: standard normal variate, Raw: spectra without pre-processing.

**Table 2-2** Characteristics of PLSR regression models for NIR and ATR-IR spectra for EC in *E. bosistoana* heartwood for different pre-processing spectra methods.  $R^2$ : coefficient of determination and RMSE: root-mean-square error. The subscripts <sub>CV</sub> and <sub>P</sub> denote model based on calibration data set using leave-one-out cross-validation and prediction when the model was applied to the validation data set, respectively. SNV: standard normal variate, Raw: spectra without pre-processing.

	Pre-treatment	Calibration			Validation	
	spectra	$R^2_{CV}$	$RMSE_{CV}$	Ncomp	$R^2_P$	$RMSE_P$
NIR	Raw (None)	0.92	0.99	5	0.83	0.8

	SNV	0.90	1.25	3	0.88	1.28
	1 <sup>st</sup> derivative	0.91	1.06	2	0.79	0.89
	2 <sup>nd</sup> derivative	0.87	1.24	3	0.68	1.08
ATR-IR	raw (NO)	0.72	1.67	3	0.72	1.38
	SNV	0.70	1.71	2	0.67	1.52
	1 <sup>st</sup> derivative	0.71	1.70	3	0.70	1.43
	2 <sup>nd</sup> derivative	0.71	1.70	3	0.70	1.44

### 2.3.3 Interpretation of PLSR models based on different pre-processing methods

Interpretation of PLSR models is difficult as the model algorithms can interfere with the correlation between the spectra frequency to the target variable (Tran et al., 2014). The sMC algorithm has been developed to facilitate PLSR model interpretation. The sMC method was used to investigate the effect of 4 different spectra pre-processing on the significant variables to predict EC in *E. bosistoana*, for both, the NIR and ATR-IR region.

Spectra pre-processing influenced the variables, which correlated to the EC (Figures 2-3 and 2-4). The figures show the average spectra after signal processing as well as the explained variance of the individual frequencies (blue solid line). Compared to the unmodified spectra similar frequencies but with different relative importance were identified by sMC after SNV normalisation. These signals also appeared sharper. An exception was observed for the region from 4785 to 4485  $\text{cm}^{-1}$ , which was not contributing after SNV normalisation. Water has a strong signal at  $\sim 5000 \text{ cm}^{-1}$  and wood is hygroscopic. It might be that the SNV normalisation of this variable region affected the neighbouring signal at 4785-4485  $\text{cm}^{-1}$ .

Transformation of spectra into their 1<sup>st</sup> derivative results in a single peak being converted in a split peak with extremes at the flanks of the original signal (Salmén and Bergström, 2009). This was reflected also in the explained variance identified with the sMC algorithm. For

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example the identified region around  $1174\text{ cm}^{-1}$  in the unmodified and SNV spectra was represented by a double peak at  $1226$  and  $1161\text{ cm}^{-1}$  or the region  $4785\text{--}4485\text{ cm}^{-1}$  as a double peak at  $4724$  and  $4520\text{ cm}^{-1}$ . For NIR spectra, the spectra regions explaining the variance were more similar to the unmodified than the SNV normalised spectra. As expected, the signals also appeared sharper as derivatives are a common means of resolving signal overlap.

2<sup>nd</sup> derivative spectra are often used to resolve signal overlap and positive signals are converted into negative peaks. The spectral regions identified by the sMC mirrored those of the unmodified spectra but with sharper and more numerous peaks.

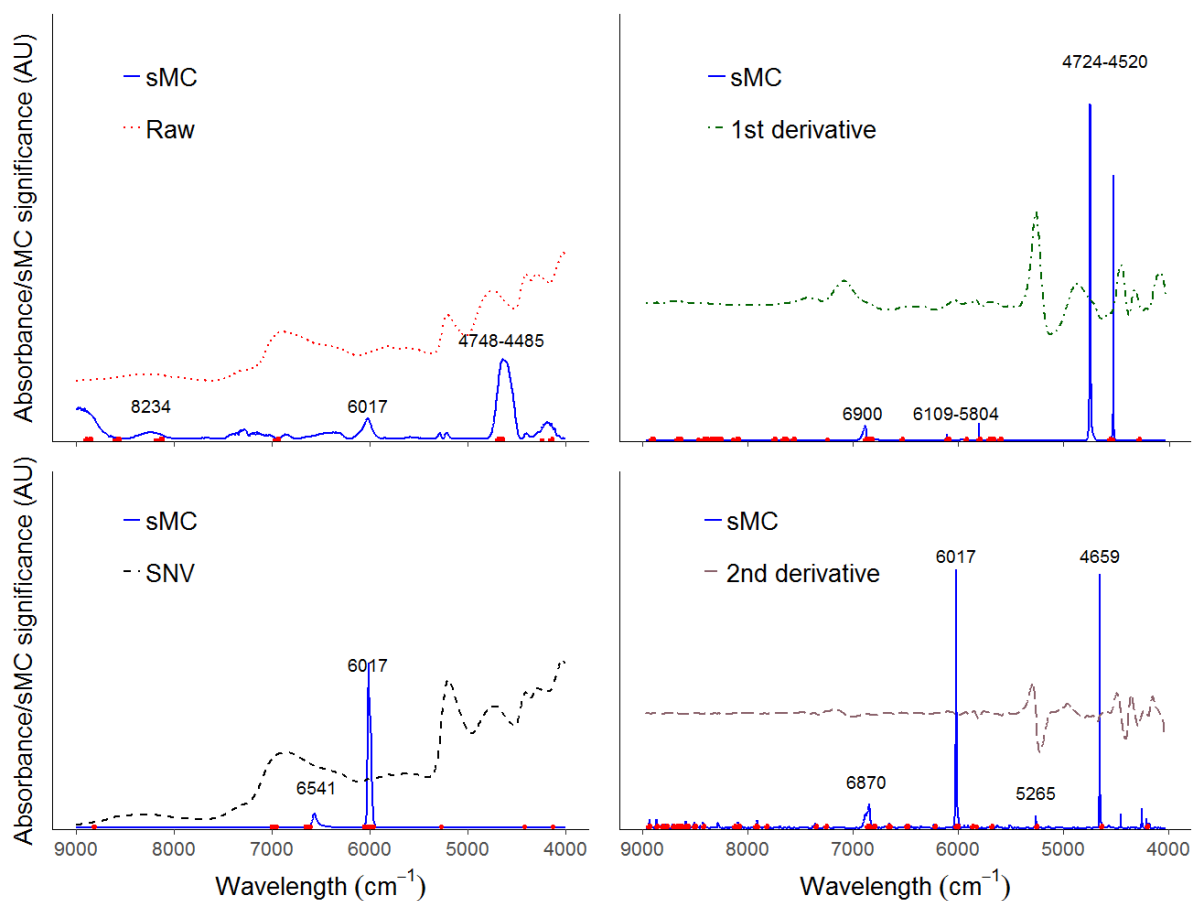
In general the signals identified by the PLSR/sMC models included those which were characteristic for heartwood extract (Figure 2-1). These include  $\sim 6900$ ,  $6017$ ,  $4659$ ,  $1712$ ,  $1330\text{--}1300$  and  $\sim 1174\text{ cm}^{-1}$ , which were associated to the phenolic O-H, C<sub>ar</sub>-H groups, ketones, esters, conjugated aldehydes and condensed tannins that occur in the heartwood extractives.

In the region from  $9000$  to  $7000\text{ cm}^{-1}$  numerous signals have been reported, for example the 1<sup>st</sup> overtones and 2<sup>nd</sup> overtones of O-H and N-H stretching vibrations as well as the 1<sup>st</sup> overtones of C-H combination bands. For example signals at  $8650$  to  $8450\text{ cm}^{-1}$  were assigned to the 1<sup>st</sup> and 2<sup>nd</sup> overtone of C-H stretching vibrations from methyl groups (Schwanninger et al., 2011).

For ATR-IR spectroscopy, without any pre-processing method, the significant variables were mainly in the region of  $1800$  to  $1400\text{ cm}^{-1}$ . Several peaks on the ATR-IR spectra were found, including the peaks at  $1787$ ,  $1650$ ,  $1440$ ,  $1300$ ,  $1174$  and  $860\text{ cm}^{-1}$ . Peaks at  $1787\text{ cm}^{-1}$  were associated with the 1<sup>st</sup> overtone of C-H stretching of CH<sub>2</sub> groups and a signal at  $1650\text{ cm}^{-1}$  was reported characteristic of aromatic skeletal C=C stretching in in extractives compounds (Faix, 1991). C-H bending was reported at around  $1440\text{ cm}^{-1}$  while the CH<sub>3</sub> stretching

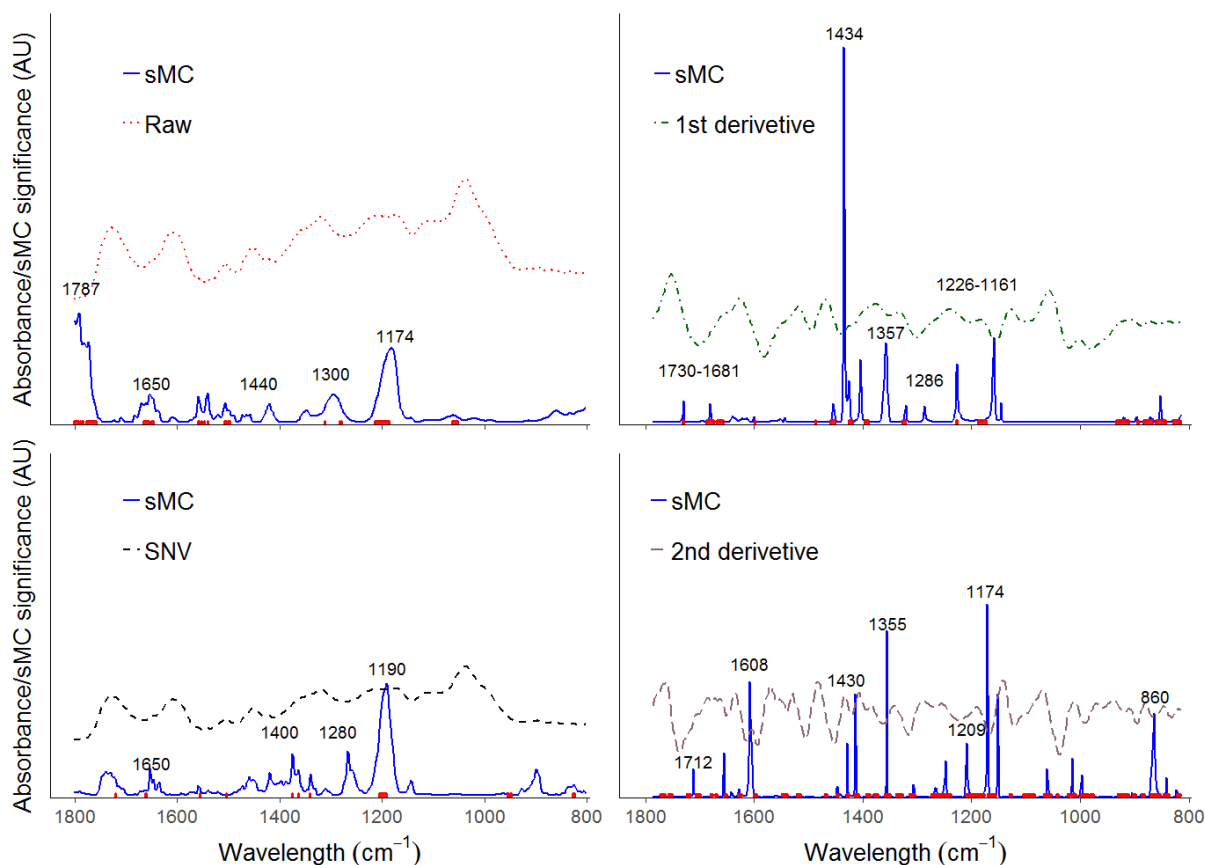
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vibrations were associated with peaks at 1317 and 1174  $\text{cm}^{-1}$  (Acquah et al., 2016; Yang et al., 2005).



**Figure 2-3 Influence of pre-processing on NIR spectra, the correlation to extractive content of *E. bosistoana*, and the variable selected by the sMC algorithm (upper left: raw spectra, upper right: 1<sup>st</sup> derivative, bottom left: SNV, bottom right: 2<sup>nd</sup> derivative). Dashed lines: average NIR spectra; blue solid lines: sMC – explained variance; red points: variables selected by the sMC algorithm; SNV: standard normal variate; Raw: spectra without pre-processing. AU: arbitrary unit.**





**Figure 2-4** Influence of pre-processing on ATR-IR spectra, the correlation to extractive content of *E. bosistoana*, and the variable selected by the sMC algorithm (upper left: raw spectra, upper right: 1<sup>st</sup> derivative, bottom left: SNV, bottom right: 2<sup>nd</sup> derivative). Dashed lines: average ATR-IR spectra; blue solid lines: sMC – explained variance; red points: variables selected by the sMC algorithm; SNV: standard normal variate; Raw: spectra without pre-processing. AU: arbitrary unit.

### 2.3.4 Variable selection

Typically, only some frequencies of IR spectra are strongly correlated to the target variable (Westad and Martens, 2000). Not all regions of the spectra contribute to the prediction models and some frequencies might even reduce the precision of the models (Mehmood et al., 2012). Consequently selecting the most relevant variable can improve model quality. Table 2-3 presents PLSR model characteristics after variable selection with the sMC algorithm (Tran et al., 2014), one of the numerous algorithms which have been proposed for variable selection (Mehmood et al., 2012). The number of variables was significantly reduced by the sMC

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algorithm for both NIR and ATR-IR PLSR while the optimal number of components remained the same as for the models based on the full spectra. Considering spectra pre-processing methods conversion of the spectra into their 1<sup>st</sup> or 2<sup>nd</sup> derivative resulted in more selected variables compared to the unmodified or baseline (SNV) corrected spectra. After variable selection, the RMSE for NIR spectra based models was reduced from 0.99 - 1.25% to 0.91 - 1.16% dependent of the spectra pre-processing. The largest reductions in RMSE were observed for the 2<sup>nd</sup> derivative spectra (Table 2-2 and 2-3). No marked improvement of the RMSE for ATR-IR spectra based models was achieved by variable selection ranging from 1.62 to 1.67% after variable selection compared to 1.67 to 1.71% for the full models.

The models based on the unmodified NIR spectra showed the best results for both the calibration and validation sets but also need the most components to reach this level. SNV normalisation reduced the model accuracy both in NIR and ATR-IR spectra (Table 2-3). The 2<sup>nd</sup> derivative spectra yielded robust and more accurate prediction results compared to the 1<sup>st</sup> derivative spectra for both, the NIR and ATR-IR technique.

The chosen variables comprised signals (Figure 2-3 and 2-4), which were related to heartwood extracts (~6900, 6017, 4659, 1730 and 1174 cm<sup>-1</sup>) (Figure 2-1) as well as others (8234 and 6541 cm<sup>-1</sup>) which might be associated with cell wall polymers. For example, the peaks at 8234 and 6541 cm<sup>-1</sup> were associated to the 2<sup>nd</sup> overtone of the C-H and 1<sup>st</sup> overtone of the O-H stretching vibrations of cellulose in wood (Fackler and Schwanninger, 2010; Jackson, 1968). Extractive content is a relative measure in respect to the cell wall material in a sample. Therefore, a calibration needs also to consider the amount of cell wall material present in the spectra; in other words, the spectra need to be normalised for the absolute amount of matter in the light beam.

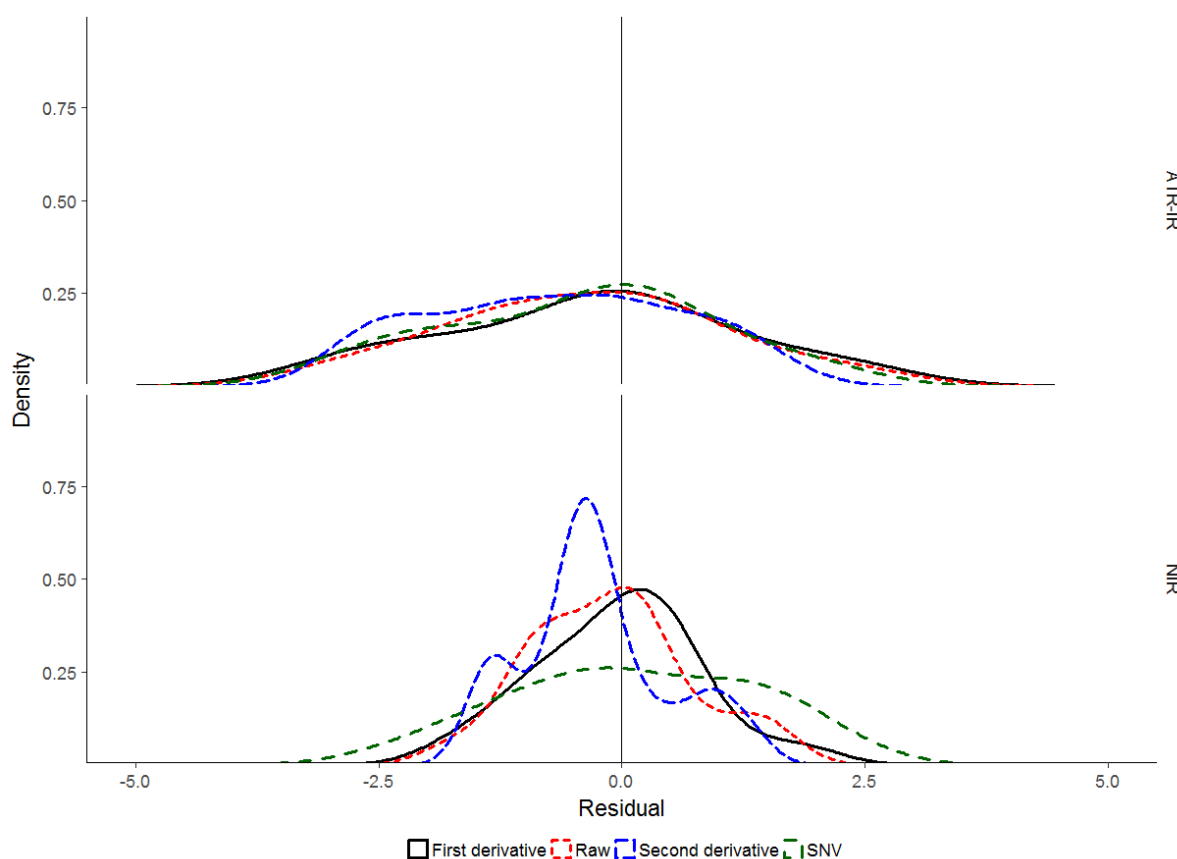
For the NIR spectra the sMC algorithm selected all three major extractive signals ( $\sim 6900$ ,  $6017$  and  $4659\text{ cm}^{-1}$ ) after 1<sup>st</sup> and 2<sup>nd</sup> derivative conversion (Figure 2-3). Interestingly only two of the three signals were selected for the unmodified ( $\sim 6900$  and  $4659\text{ cm}^{-1}$ ) and SNV normalised spectra ( $\sim 6900$  and  $6017\text{ cm}^{-1}$ ). For ATR-IR spectra, extract specific signals including  $1730$ ,  $1174$  and  $860\text{ cm}^{-1}$  were selected by the sMC algorithm. However, also a relatively large proportion of unidentified signals was selected potentially also including cell wall signals (Figure 2-4).

**Table 2-3 NIR and ATR-IR PLSR regression models based on different pre-processing spectra for calibration and validation of EC with sMC variable selection.  $R^2_{CV}$ : coefficient of determination of cross-validation.  $R^2_P$ : coefficient of determination of prediction when the model is applied to validation data set, respectively;  $RMSE_{CV}$ : root-mean-square error of cross-validation.  $RMSE_P$ : root mean-square error of prediction when the model is applied to validation data set. SNV: standard normal variate, Raw: spectra without any pre-processing.**

Spectra	Total number of variables	Pre-treatment	Variables selected by sMC	Calibration			Validation	
				$R^2_{CV}$	$RMSE_{CV}$	Ncomp	$R^2_P$	$RMSE_P$
NIR	1296	Raw (NO)	37	0.93	0.91	5	0.82	0.81
	1296	SNV	71	0.92	1.16	3	0.86	1.47
	1282	1 <sup>st</sup> derivative	137	0.91	1.06	2	0.81	1.58
	1282	2 <sup>nd</sup> derivative	84	0.91	1.03	3	0.82	0.84
ATR-IR	520	Raw (NO)	64	0.72	1.65	3	0.71	1.42
	520	SNV	23	0.72	1.66	2	0.7	1.43
	506	1 <sup>st</sup> derivative	85	0.72	1.67	3	0.67	1.51
	506	2 <sup>nd</sup> derivative	166	0.73	1.62	3	0.72	1.40

The density plots of the residuals between measured and predicted EC values of *E. bosistoana* heartwood using the sMC-PLSR NIR and ATR-IR models are displayed in Figure 2-5. The residuals of the validation data sets visualise the accuracy (bias) and precision

(deviation around the mean). Both, the NIR and ATR-IR based models gave accurate predictions with no obvious bias. The NIR models were more precise than ATR-IR model having smaller residuals, similar results were found by Zhou et al. (2015).



**Figure 2-5** Density plot of predicted EC (%) residuals of *E. bosistoana* heartwood by NIR model and ATR-IR model with selected wavenumbers in the validation sets based on 2<sup>nd</sup> derivative spectra. SNV: standard normal variate, Raw: spectra without any pre-processing.

## 2.4 Conclusions

NIR and ATR-IR PLSR regression models were able to predict the EC in heartwood of *E. bosistoana* from wood powder samples and can serve as fast and non-destructive methods for tree breeders to screen eucalyptus for heartwood quality. Both, NIR and ATR-IR based models were accurate with no bias but NIR based models were with more precise. Variables selection with the sMC algorithm improved the precision of the NIR based models to 0.9%, a useful result considering the EC range of 0.96% to 14.67% in the heartwood of 7 year-old *E.*

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*bosistoana*. Variable selection had no benefit on the accuracy of ATR-IR spectra. Spectra pre-processing methods did not offer substantial advantages for both the NIR and ATR-IR spectra. However, 2<sup>nd</sup> derivative transformation of NIR spectra combined with variables selection reduced the required number of components compared to unmodified spectra. The PLSR models selected extractive signals that related to chemical structures known of heartwood extracts including ~6900 (phenolic O-H groups), 6017 (C<sub>ar</sub>-H groups), 4659 (C<sub>ar</sub>-H and C=O groups), 1330-1300 (phenolic O-H groups), and ~1174 cm<sup>-1</sup> (condensed tannins).

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## Chapter 3 **Predicting extractives content of *Eucalyptus bosistoana* F. Muell. heartwood from stem cores by near infrared spectroscopy**

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### **3.1 Introduction**

Wood is a biodegradable material (Hillis, 1987; Scheffer and Cowling, 1966; Taylor et al., 2007). While biodegradability is a positive attribute, premature degradation while wood is in service is a problem. Not all wood is equally prone to biodegradation. Durability describes the resistance of wood to biological decay by fungi, insects or marine borers. Secondary metabolites, so-called extractives, deposited by trees into the stem at a later age are a main factor determining the natural durability of wood (Hawley et al., 1924). Wood containing these extractives is called heartwood and is found at the centre of stems. Removing extractives from a durable heartwood allows it to decay while adding heartwood extractives to non-durable wood will enhance decay resistance (Smith et al., 1989). Therefore, the amount of extractives in heartwood largely determines the degree of natural durability (Aloui et al., 2004; Hart, 1981; Hart and Hillis, 1972; Haupt et al., 2003).

Natural durability is highly variable within a species. For example durability ratings were reported to vary from very-durable (Class 1) to non-durable (Class 3) for sessile oak (Brischke et al., 2009). This variation is partly under genetic control (Bush et al., 2011; Poke

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et al., 2006). To ensure a quality timber product of high durability, it is necessary to reduce this variation. This can be achieved for future plantations by selection in a tree breeding program. For example in *Eucalyptus cladocalyx* natural durability was shown to have moderate-high narrow sense heritability and no adverse correlation to tree growth (Bush et al., 2011). Direct assessments of durability that rely on measuring the mass loss of wood samples in laboratory tests or field trials are time- and cost-consuming, running over months or even decades (EN 350-1, 1994). They are not suitable for cost-efficient tree breeding programs, which rely on the assessment of a large number of individuals in a timely manner (Harju and Venäläinen, 2006). Thus, there is a need to replace these tests with a rapid and cost effective analytical method. Since extractive levels are closely correlated to decay resistance [12], the extractive content (EC) can be considered a proxy for natural durability. Quantifying extractives in heartwood using Soxhlet or accelerated solvent extraction (ASE) is time consuming and requires sample milling (Tappi, 2004).

Near infrared (NIR) spectroscopy is a non-destructive technique that is used for the chemical analysis of agricultural and medical products (Forina et al., 2015; Guillemain et al., 2017; Malegori et al., 2017). Acquiring NIR spectra is fast, only taking seconds. The method has been exploited in tree breeding for wood properties on milled and solid samples (Jones et al., 2008; Schimleck et al., 2005; Tsuchikawa and Schwanninger, 2013). Challenges for using NIR for the quantification of the chemical composition of wood are the confounding influences of moisture (Giordanengo et al., 2008) and sample form (Hein et al., 2010). Fresh samples from standing trees frequently have moisture contents (MC) over 100%, dominating the spectra due to the high sensitivity of the technique to O-H vibrations (Leblon et al., 2013). Therefore, wood samples are usually air-dried before NIR analysis (Giordanengo et al., 2008). However, wood is a hygroscopic material and requires tight MC control, especially when measuring samples in powder form or at the surface, which quickly equilibrate to the

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surrounding condition. This requires controlled environment facilities that are often unavailable in commercial settings. The  $R^2$  of NIR calibrations for phenol content of wood were reported to decrease from 0.77 at constant MC of 9% to 0.02 when MC varied between 0 and 21% (Giordanengo et al., 2008). Particle size in case of milled wood (Hein et al., 2010) as well as surface roughness and the anatomical face, i.e. radial, tangential or transverse in case of solid wood samples, were reported to influence NIR spectra and calibrations (Braga et al., 2011; Schimleck et al., 2007). For example, the  $R^2$  for predicting lignin content in eucalyptus wood by NIR varied between 0.7 and 0.9 for solid wood samples with different surface roughnesses and particle sizes (Hein et al., 2010). The grain angle was predicted by NIR with a root mean square error of 6 ° (Gindl and Teischinger, 2007).

NIR spectroscopy has been used to determine the quantity of heartwood extractives in some tree species using wood powder, achieving an accuracy (residual mean square error - RMSE) of 0.5 to 2% (Ribeiro da Silva et al., 2013; Taylor et al., 2011). Employing NIR spectroscopy in a breeding program, which relies on the assessment of thousands of trees, could benefit from minimising sample preparation. Therefore, it is desirable to develop a NIR spectroscopy methodology to directly assess solid wood samples with varying MCs. Furthermore, accurately the quantifying heartwood extractives in solid wood would allow to assess the spatial distribution of extractives in stems. Data processing algorithms such as External Parameter Orthogonalisation (EPO) (Roger et al., 2003) or significant Multivariate Correlation (sMC) (Tran et al., 2014) can remove confounding effects on NIR calibrations (Barreiro et al., 2008; Minasny et al., 2011; Wijewardane et al., 2016).

*E. bosistoana* is the target species of the New Zealand Dryland Forests Initiative (NZDFI) that aims to establish an alternative plantation forest industry in New Zealand to provide a sustainable supply of ground durable timber (Altaner et al., 2017). The species has been chosen from 25 durable eucalypt species, which have been trialled in New Zealand over the

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last decades (Van Ballekom and Millen, 2017). *E. bosistoana* is a class 1 durable timber with a life expectancy of >25 years in ground, has excellent mechanical properties (Bootle, 2005) and has growth characteristics suitable to New Zealand's conditions. A central part of the New Zealand based project is a breeding program, based on a seed collection of >200 families covering the native distribution of this previously undomesticated species, to ensure the industry is based on fast-growing, healthy trees producing quality timber.

The objective of this work was to develop a fast and robust methodology to quantify the extractive content in heartwood of *E. bosistoana* for future use in our breeding program based on 10.000s of trees. The potential for data processing algorithms to realise time and cost savings by reducing requirements for sample preparation was evaluated. In particular, the effects of MC, grain direction and sample form were investigated. Finally, the data processing algorithms were tested to determine if the effect of local EC variations on NIR calibrations of solid wood could be removed.

## **3.2 Materials and Methods**

### **3.2.1 Materials**

*Eucalyptus bosistoana* F. Muell trees were planted in 2009 in Marlborough, New Zealand at two sites (41 °26'S, 173 °56'E and 41 °43'S, 174 °02'E) in a dry subtropical climate, with an average annual rainfall of approximately 1000 mm (Altaner et al., 2017). The trials were part of a tree breeding program representing 67 open-pollinated families planted in 2.4 m ×1.8 m spacing as 150 incomplete, single-tree plots with 35 trees per plot.

Stem discs used for this study originated from a thinning operation of the trials in December 2015. One hundred-and-twenty-six trees which contained heartwood were used for this study. Stem cores were obtained from 47 of the remaining trees in the same trials in May 2016.

#### **3.2.1.1 Discs**

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Approx. 100 mm long stem sections were cut from the base of the trees. Twenty to thirty mm thick discs were cut from 70 randomly selected trees and sawn in half through the pith with a band saw to expose a longitudinal-radial face. Samples were first conditioned at 25 °C and 60% relative humidity to obtain a stable MC (~9%). Air-dry NIR spectra were obtained, then the wood was oven-dried at 60 °C (<2% MC). Samples were allowed to cool in a desiccator before collecting oven-dry spectra.

Heartwood was isolated from the remaining 70-80 mm long stem sections by drilling into the transverse face with a 12 mm drill. The drill shavings were further milled in a Wiley mill fitted with a 2 mm screen.

#### **3.2.1.2 Cores**

Full diameter stem cores with a core diameter of 14 mm were obtained at a height of approx. ~50 cm of *E. bosistoana* trees. Eleven cores were used in the grain angle assessments. A further 36 cores were used to validate the EPO-PLS model for extractive content (EC) by first acquiring NIR spectra and then milling the heartwood for extraction.

#### **3.2.1.3 Determination of Extractives content (EC)**

Between 5-8 g of oven-dry wood powder was weighed into 33 ml stainless steel cells and extracted with 100% ethanol in an accelerated solvent extraction system (ASE) (Thermo Scientific) using the following machine settings: static time 15 min, temperature 70 °C, 100% rinse volume and 2 extraction cycles. The mass of extractives in the ethanol solution was determined after evaporating the solvent followed by oven-drying at 105 °C. The EC was calculated as the mass of extract in relation to the unextracted oven-dry wood mass.

#### **3.2.2 NIR spectroscopy**

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NIR spectra were taken from discs and cores with a fibre optic probe (Model N-500, Bruker) at wavelengths from 9000 to 4000  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  intervals. Thirty two scans were averaged for each spectrum.

### ***3.2.2.1 NIR spectra of milled wood***

Before extraction, NIR spectra of all 126 milled wood samples were collected in air-dry and oven-drying conditions. Each milled wood powder was sampled three times and the spectra were averaged.

### ***3.2.2.2 NIR spectra of disc***

NIR spectra were collected from both the radial-longitudinal and the transverse face of the wood discs in air-dry and oven-dry conditions. NIR spectra of heartwood were taken at 5 mm intervals from pith to bark. The extractive content was predicted for each radial position and subsequently weight averaged for the area they represented on the disc. In total, NIR spectra were collected from 126 discs from the transverse face and a subset of 70 of these discs from the radial face.

### ***3.2.2.3 NIR spectra of cores***

Eleven cores were used to investigate the influence of grain angle on NIR spectra. Cores ranged between 76 and 240 mm in length with a heartwood diameter of 40 to 135 mm. Spectra were collected at 5 mm intervals along the core in the heartwood at 0°, 45°, 90°, 135°, 180°, 225°, 270° and 315° in respect to the stem axis (Figure 3-1). Due to symmetry, spectra for 0° and 180°, 45°, 135°, 225° and 315°, as well as 90° and 270° were averaged, respectively. Sapwood spectra were also collected at all angles at one radial position at each core end.

NIR spectra of heartwood were collected at 5 mm intervals on the transverse face of a further 36 cores.



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The extractive content was predicted for each radial position and subsequently averaged for a core.

### 3.2.3 Analysis

Data were analysed in R (Team, 2014). The prospectr package (Stevens and Ramirez-Lopez, 2014) was used for NIR spectra manipulation including standard normal variate (SNV), multiple scatter correction (MSC) and conversion into the 1<sup>st</sup> and 2<sup>nd</sup> derivatives with the Savitzky-Golay algorithm using a window size of 15 data points. The plsVarSel package (Mehmood et al., 2012) was used for significant Multivariate Correlation (sMC) and the pls package (Mevik et al., 2015) using the pls algorithm with leave-one-out cross-validation was used to develop calibration models. Tukey's test was used to compute the significant difference between grain directions.

#### 3.2.3.1 External parameter orthogonalisation (EPO)

EPO was developed to remove the effect of temperature from NIR spectra of fruit (Roger et al., 2003) and successfully used to remove the effect of moisture on NIR spectra of wood powder (Giordanengo et al., 2008). A minimum of 60 samples was recommended for EPO. Detail of the EPO algorithm has already been published (Giordanengo et al., 2008; Minasny et al., 2011). In short, the domain in the spectra representing the chemical information independent of an external parameter is separated from the effects of the external parameter and the residuals, and subsequently used for modelling.

#### 3.2.3.2 Variable selection

Variable selection is computationally demanding and various algorithms have been suggested (Mehmood et al., 2012; Tran et al., 2014). The sMC algorithm was used, which chooses the wavenumbers with minimal false negative and false positive error and concurrently highlights the variables with the strongest correlation to the response (Tran et al., 2014).

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### 3.2.3.3 *Data sets*

Several data sets were used for different aspects of this study (Table 3-1).

EC model data set:

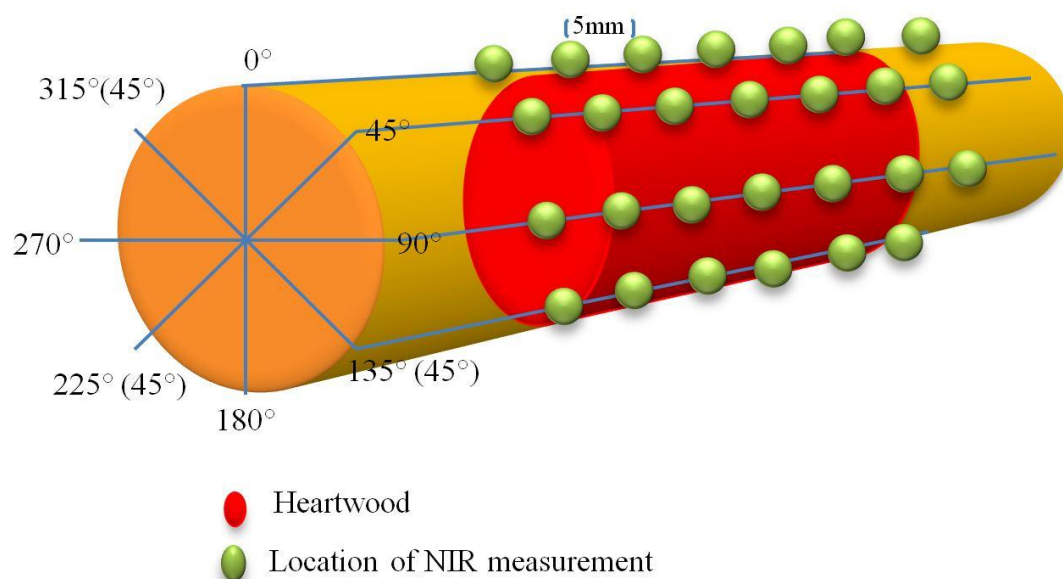
All 126 samples were randomly divided into two data sets, one with 106 samples for calibration and the other with 20 samples for validation of the model. These data sets contained NIR spectra collected from both discs (transverse face) and powders in air-dry condition (used in 3.3.2, 3.3.4, 3.3.5 and 3.3.6).

EPO-data set:

A subset of 70 stem discs for which NIR spectra were collected in air-dry and oven-dry conditions from both the radial and the transverse face, as well as after milling in the air-dry condition were used for EPO transformation for MC, grain direction and sample form (used in 3.3.1, 3.3.2, 3.3.3 and 3.3.5).

Grain-validation data set:

NIR spectra collected on 11 air-dry stem cores at the transverse and radial faces as well as the faces oriented at 45 °between these (Figure 3-1) were used to investigate the effect of grain direction (used in 3.3.2).



**Figure 3-1 Sampling strategy on the cores**

Core-validation data set:

NIR spectra taken from the transverse face of 36 air-dried stem cores were used to validate the developed methodology. After collecting NIR spectra on the transverse face of the cores in 5 mm intervals along the heartwood, the heartwood of these cores was milled and extracted with ethanol to determine the extractive content (used in 3.3.6).

**Table 3-1 Summary statistics of ethanol soluble extractive content (EC) in heartwood of *E. bosistoana* for the used data sets. CV: Coefficient of variation.**

EPO		EC model		Core validation
(n=70)	Calibration	Validation		(n=36)

		(n=106)		(n=20)	
EC (%)	Max	14.67	14.98	14.92	14.79
	Mean	4.39	6.46	7.02	8.46
	Min	1.32	1.32	2.73	2.01
	CV	0.45	0.52	0.24	0.28

### 3.3 Results and discussion

#### 3.3.1 Preliminary data analysis

The ethanol soluble EC of the *E. bosistoana* heartwood ranged between 1.3 and 15.0% (Table 3-1). NIR spectra from the transverse face of wood discs in oven-dry condition gave the most accurate models for EC compared to those using spectra from the radial face or at air-dry condition (Table 3-2). Different pre-processing methods were applied to the NIR spectra from the wood discs before building PLS regression models with EC. The most accurate models to predict EC from the solid wood NIR spectra were obtained after standard normal variate (SNV) transformation and subsequent conversion into the 1<sup>st</sup> derivative. This improved the accuracy by ~25%. For the following analysis of EC all spectra were converted to the 1<sup>st</sup> derivative after SNV transformation.

**Table 3-2 Performance of PLS regression models for EC in heartwood of *E. bosistoana* based on spectra having undergone different pre-processing making use of the EPO data set.  $R^2_{CV}$ : coefficient of determination (cross-validation);  $RMSE_{CV}$ : root mean square error (cross-validation); Ncomp: number of components in the PLS model. Values in columns represent spectra of the following conditions: Transverse, air-dry / Transverse, oven-dry / Radial, air-dry / Radial, oven-dry.**

Spectra	Pre-treatment	$R^2_{CV}$	$RMSE_{CV}$	Ncomp
Transverse, air-dry	No (raw spectra)	0.65 / 0.74 / 0.69 / 0.64	1.76 / 1.54 / 1.65 / 1.75	4 / 6 / 4 / 4
/	SNV	0.64 / 0.75 / 0.58 / 0.61	1.78 / 1.50 / 1.92 / 1.84	3 / 4 / 2 / 3
Transverse, oven-dry	1 <sup>st</sup> derivative	0.65 / 0.73 / 0.70 / 0.64	1.77 / 1.58 / 1.62 / 1.77	3 / 4 / 3 / 3

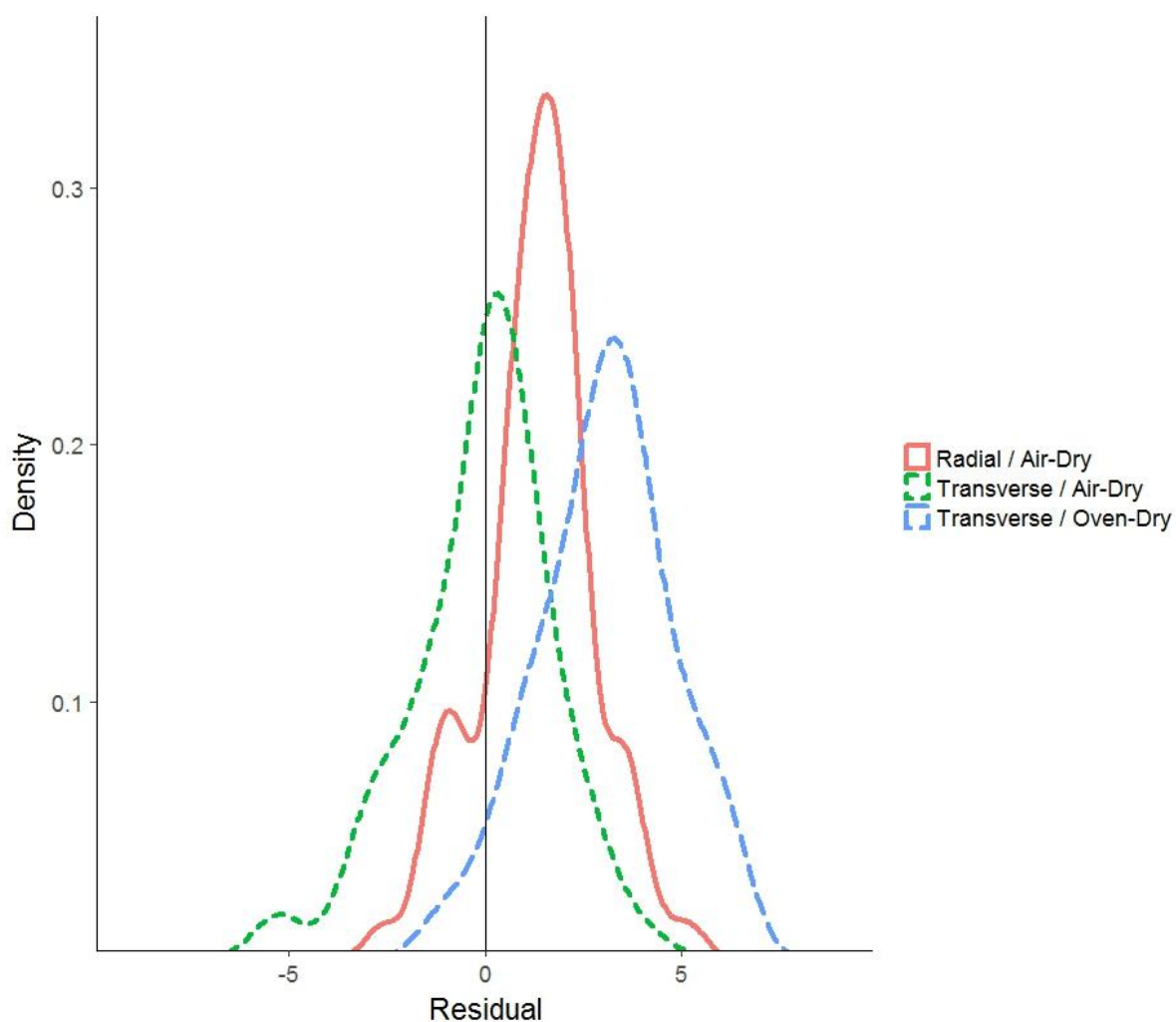
/	2 <sup>nd</sup> derivative	0.56 / 0.68 / 0.65 / 0.59	1.97 / 1.70 / 1.76 / 1.87	3 / 5 / 3 / 4
Radial, air-dry	MSC	0.64 / 0.75 / 0.69 / 0.61	1.78 / 1.50 / 1.63 / 1.84	3 / 4 / 3 / 3
/	SNV+1 <sup>st</sup> derivative	0.67 / 0.76 / 0.69 / 0.61	1.60 / 1.48 / 1.63 / 1.84	3 / 3 / 2 / 3
Radial, oven-dry	SNV+2 <sup>nd</sup> derivative	0.59 / 0.69 / 0.65 / 0.59	1.92 / 1.67 / 1.76 / 1.89	2 / 5 / 3 / 3

### 3.3.2 The effect of MC and grain direction on EC models

The EC could be predicted with a  $RMSE_{CV}$  of 1.45% (Table 3-3) using the NIR spectra obtained from the transverse face of air-dry discs. A bias of EC of 2-5% was observed for this model (Figure 3-2) when altering the MC to oven-dry or the grain orientation to the radial face. This bias was relevant considering the range of EC in the samples (1.3-15.0%) and the difficulty of experimentally controlling MC and grain direction on the wood cores. In particular, the grain direction in the cores was uncertain, either due to the difficulty of locating the ‘up and down’ orientation of the tree in the round core or due to grain variation inherent to the tree (Harris, 1989).

**Table 3-3 Performance of a PLS regression model for EC in heartwood of *E. bosistoana* based on NIR spectra taken from air-dry discs on the transverse face using the EC model data set.  $R^2_{CV}$ ,  $R^2_P$ : coefficient of determination - cross-validation (<sub>CV</sub>) and coefficient of determination of prediction when the model is applied to validation data set (<sub>P</sub>);  $RMSE_{CV}$ ,  $RMSE_P$ : root mean square error - cross-validation (<sub>CV</sub>) and root mean-square error of prediction when the model is applied to validation data set (<sub>P</sub>); Ncomp: number of components in the PLS model.**

Spectra	Pre-treatment	Calibration			Validation	
		$R^2_{CV}$	$RMSE_{CV}$	Ncomp	$R^2_P$	$RMSE_P$
Transverse, air-dry	SNV+1 <sup>st</sup> derivative	0.83	1.45	10	0.93	0.91



**Figure 3-2** Density plot of predicted EC (%) of *E. bosistoana* heartwood residuals using the EPO data set for a PLS model built from NIR spectra taken from the transverse face in air-dry condition (green). Residuals when using this model to predict EC from spectra taken from the same samples on the transverse face in the oven-dry state (blue) or on the radial face in air-dry condition (red).

The influence of grain direction and MC on NIR calibrations make it useful to test if these factors can be predicted from NIR spectra. Different pre-processing methods did not promote a robust model for grain direction (Table 3-4). The  $RMSE_{CV}$  varied between  $5.99^{\circ}$  to  $9.59^{\circ}$  for the different pre-processing methods. This precision was in accordance with that reported for NIR calibration of grain direction for larch wood. As grain angle in wood is typically below  $10^{\circ}$  (Harris, 1989), NIR was of limited use for the determination of grain angle in wood. However, it was possible to distinguish between the transverse and the tangential faces

of the wood samples, a result similar to previous reports (Gindl and Teischinger, 2007). MC was accurately predicted ( $RMSE_{CV} < 0.5\%$ ) by NIR spectroscopy regardless of the pre-processing of the spectra. Water strongly absorbs in the NIR region and its measurement by NIR spectroscopy is routinely used for biomaterials in industrial processes (Büning-Pfaue, 2003; ElMasry et al., 2011; Inagaki et al., 2008; Jensen et al., 2006).

**Table 3-4 Performance of PLS regression models for grain angle and MC for *E. bosistoana* based on spectra having undergone different pre-processing making use of the EPO data set.  $R^2_{CV}$ : coefficient of determination (cross-validation);  $RMSE_{CV}$ : root mean square error (cross-validation); Ncomp: number of components in the PLS model.**

Property	Pre-treatment	$R^2_{CV}$	$RMSE_{CV}$	Ncomp
		air-dry / oven-dry	air-dry / oven-dry	air-dry / oven-dry
Grain Angle	No (raw spectra)	0.95 / 0.95	9.59 / 10.25	14 / 15
	SNV	0.96 / 0.95	8.48 / 9.86	17 / 15
	1 <sup>st</sup> derivative	0.96 / 0.96	8.81 / 8.83	14 / 15
	2 <sup>nd</sup> derivative	0.98 / 0.98	5.99 / 7.16	20 / 14
	MSC	0.96 / 0.95	8.60 / 10.36	12 / 13
	SNV+1 <sup>st</sup> derivative	0.97 / 0.96	7.60 / 8.79	20 / 15
	SNV+2 <sup>nd</sup> derivative	0.98 / 0.96	6.45 / 8.70	19 / 12
		transverse / radial	transverse / radial	transverse / radial
MC	No (raw spectra)	0.98 / 0.99	0.46 / 0.37	13 / 13
	SNV	0.98 / 0.99	0.48 / 0.25	12 / 9
	1 <sup>st</sup> derivative	0.98 / 0.99	0.45 / 0.37	14 / 11

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2 <sup>nd</sup> derivative	0.97 / 0.99	0.55 / 0.33	8 / 12
MSC	0.99 / 1.00	0.33 / 0.25	11 / 15
SNV+1 <sup>st</sup> derivative	0.99 / 1.00	0.34 / 0.25	9 / 14
SNV+2 <sup>nd</sup> derivative	0.99 / 0.99	0.38 / 0.28	7 / 12

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### 3.3.3 Removing the effects of grain direction and MC

The EPO algorithm was used to transform the spectra in an attempt to remove the influence of MC and grain direction. The EPO procedure was optimised by choosing the correct dimensions and PLS factors for both the influence of MC as well as grain direction. This was done with the EPO data set. The optimal (i.e. lowest RMSE) number of dimensions  $c$  and PLS factors  $h$  were found to be 2 and 3, for both, MC and grain direction, respectively (Figure 3-3). When spectra were transformed with the EPO algorithm using the optimised parameters, the bias of the predicted EC caused by MC and grain direction was removed as can be seen from the distribution of the residuals (Figure 3-4).



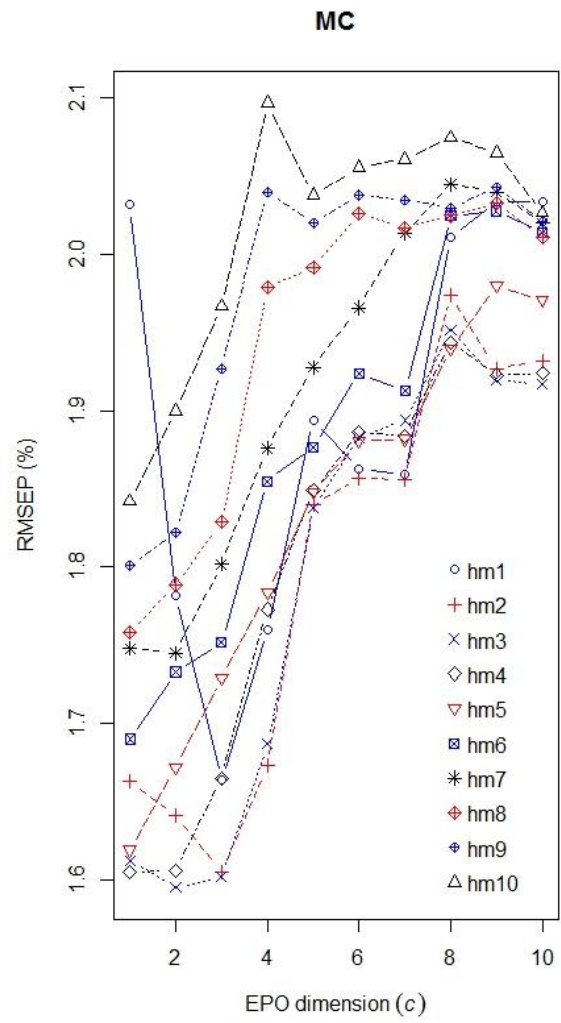
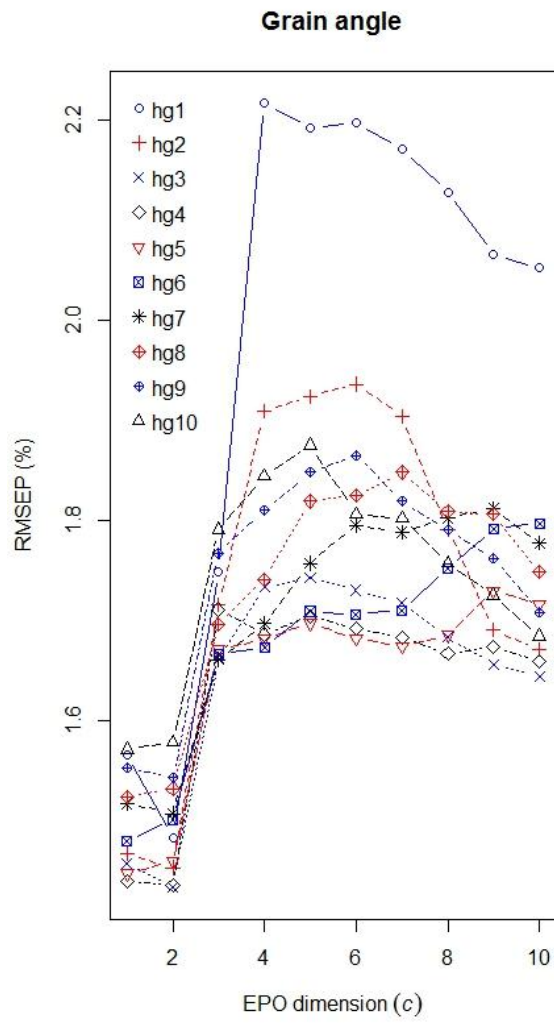


Figure 3-3 Root mean square error of prediction ( $RMSEP$ ) of EC (%) in heartwood of *E. bosistoana* for the EPO data set when transforming the spectra with the EPO-algorithm for grain (left) and MC (right) using different EPO dimensions ( $c$ ) and PLS factors ( $h$ ).

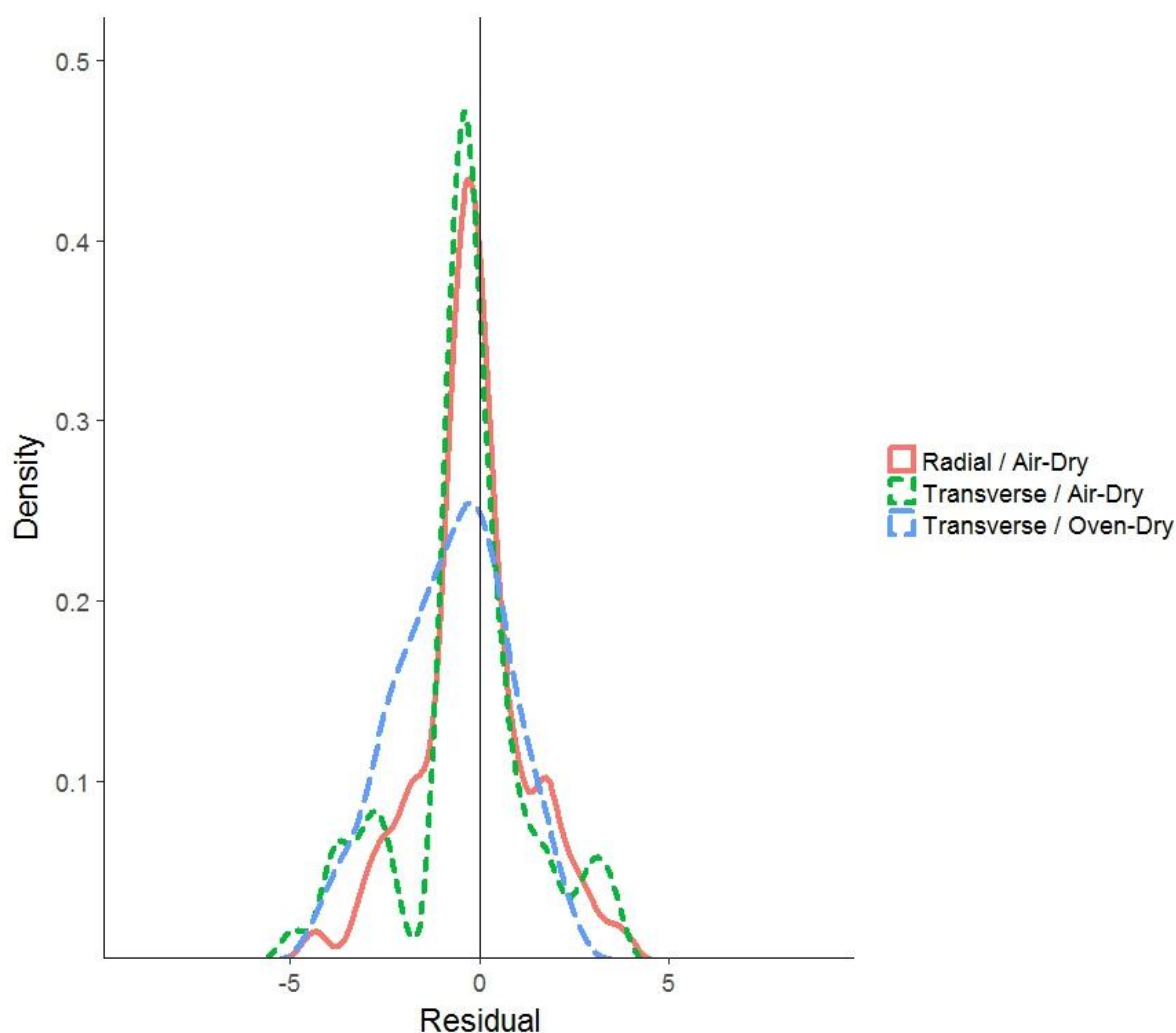


Figure 3-4 Density plot of predicted EC (%) of *E. bosistoana* heartwood residuals using the EPO data set for a PLS model build from NIR spectra taken on the transverse face in air-dry condition after EPO transformation (green). Residuals when using this model to predict EC from spectra taken from the same samples on the transverse face in the oven-dry state (blue) or on the radial face in air-dry condition (red).

This EPO-PLS model was compared to the simple PLS model (Table 3-3) by predicting EC of 11 *E. bosistoana* air-dry cores. A statistically significant difference was found between the EC measured at 0 ° (transverse face) and 90 ° (radial face) grain direction when using the PLS model without EPO transformation (Table 3-5). EPO transformation removed the effect of grain directions. No statistical significant difference was observed for 45 ° grain angle.

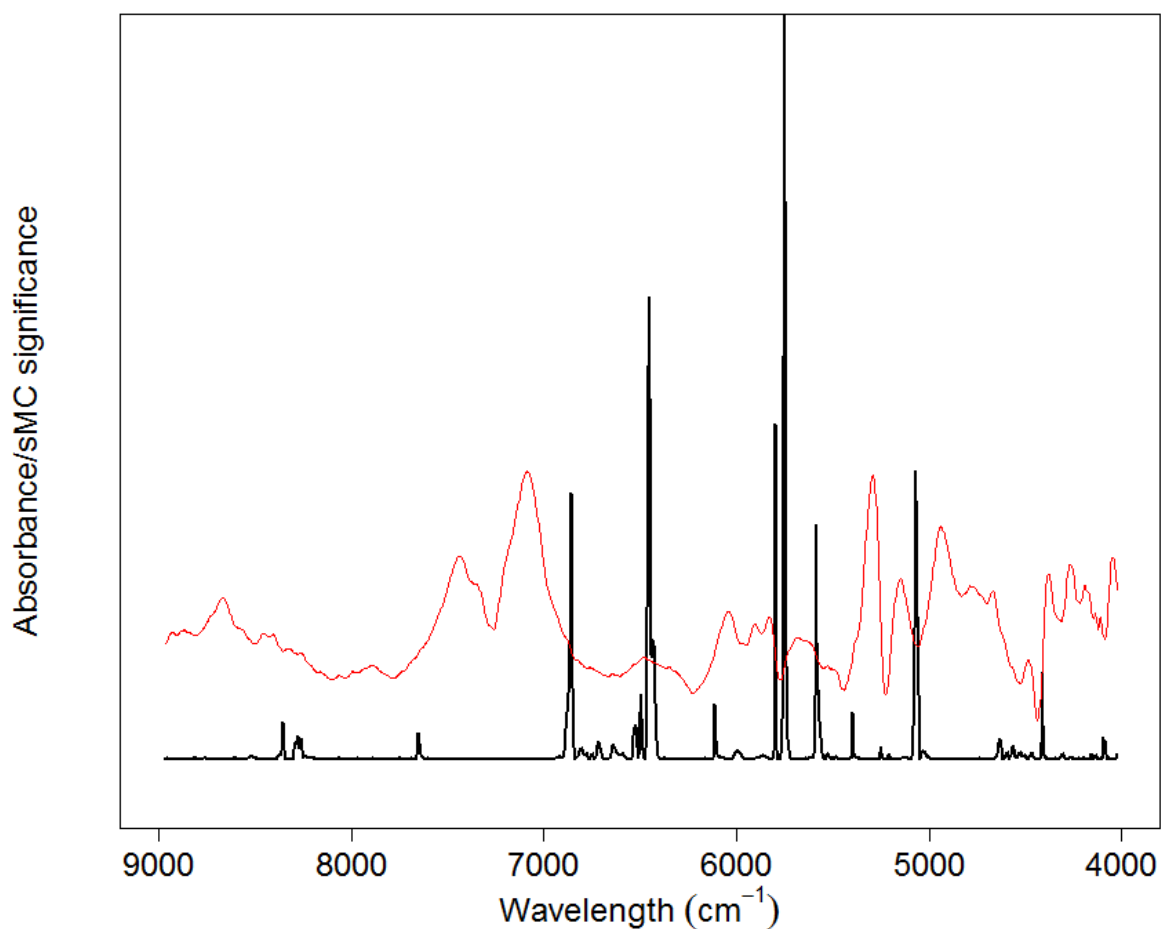
**Table 3-5 Effect of grain direction on the prediction of EC in heartwood of stem cores of *E. bosistoana*. Summary of Tukey's test between NIR spectra taken at different grain directions using the grain validation data set. Transverse face (0 °); radial face (90 °) and in-between the two faces (45 °). \*Significance at P <0.05.**

	P-value	
	PLS (without EPO)	EPO-PLS
45 °-0 °	0.26	0.99
90 °-0 °	0.03*	0.61
90 °-45 °	0.37	0.59

### 3.3.4 Variable selection

NIR models can be improved by selecting the most relevant wavenumbers for the models. NIR spectra contain signals that correlate with heartwood extractives, but also contain regions with irrelevant information such as moisture, grain direction or noise. Better models for quantitative analysis can be obtained if unimportant or confounded variables are removed from the NIR spectra.

NIR signals that significantly correlated with EC independent of MC and grain direction according to the sMC algorithm were mainly observed between 7000 cm<sup>-1</sup> and 4000 cm<sup>-1</sup>, especially at 5080 cm<sup>-1</sup> and 5750 cm<sup>-1</sup> (Figure 3-5). The 5750 cm<sup>-1</sup> band should be related to the 1<sup>st</sup> overtone of C-H stretching vibrations, while the 5080 cm<sup>-1</sup> is between symmetric and asymmetric O-H stretching vibrations coupled to O-H deformations of water (Schwanninger et al., 2011).



**Figure 3-5** Average 1<sup>st</sup> derivative NIR spectrum of *E. bosistoana* heartwood after EPO transformations (red) and the significance of variables identified by the sMC algorithm (black).

Only 173 of the 1282 wavenumbers, ~15% of the total, were identified to contribute positively to the accuracy of a PLS model for EC ( $\alpha = 0.05$ ). The accuracy of the model was improved after focusing the model on these wavenumbers with the RMSE dropping from 1.48% to 1.27% (Table 3-6).

**Table 3-6 Performance of EPO-PLS regression models for EC in heartwood of *E. bosistoana* before and after variable selection with the sMC algorithm using the EC model data sets after EPO transformation.  $R^2_{CV}$ ,  $R^2_P$ : coefficient of determination - cross-validation <sub>(CV)</sub> and coefficient of determination of prediction when the model is applied to validation data set <sub>(P)</sub>;  $RMSE_{CV}$ ,  $RMSE_P$ : root mean square error - cross-validation <sub>(CV)</sub> and root mean-square error of prediction when the model is applied to validation data set <sub>(P)</sub>; Ncomp: number of components in the PLS model.**

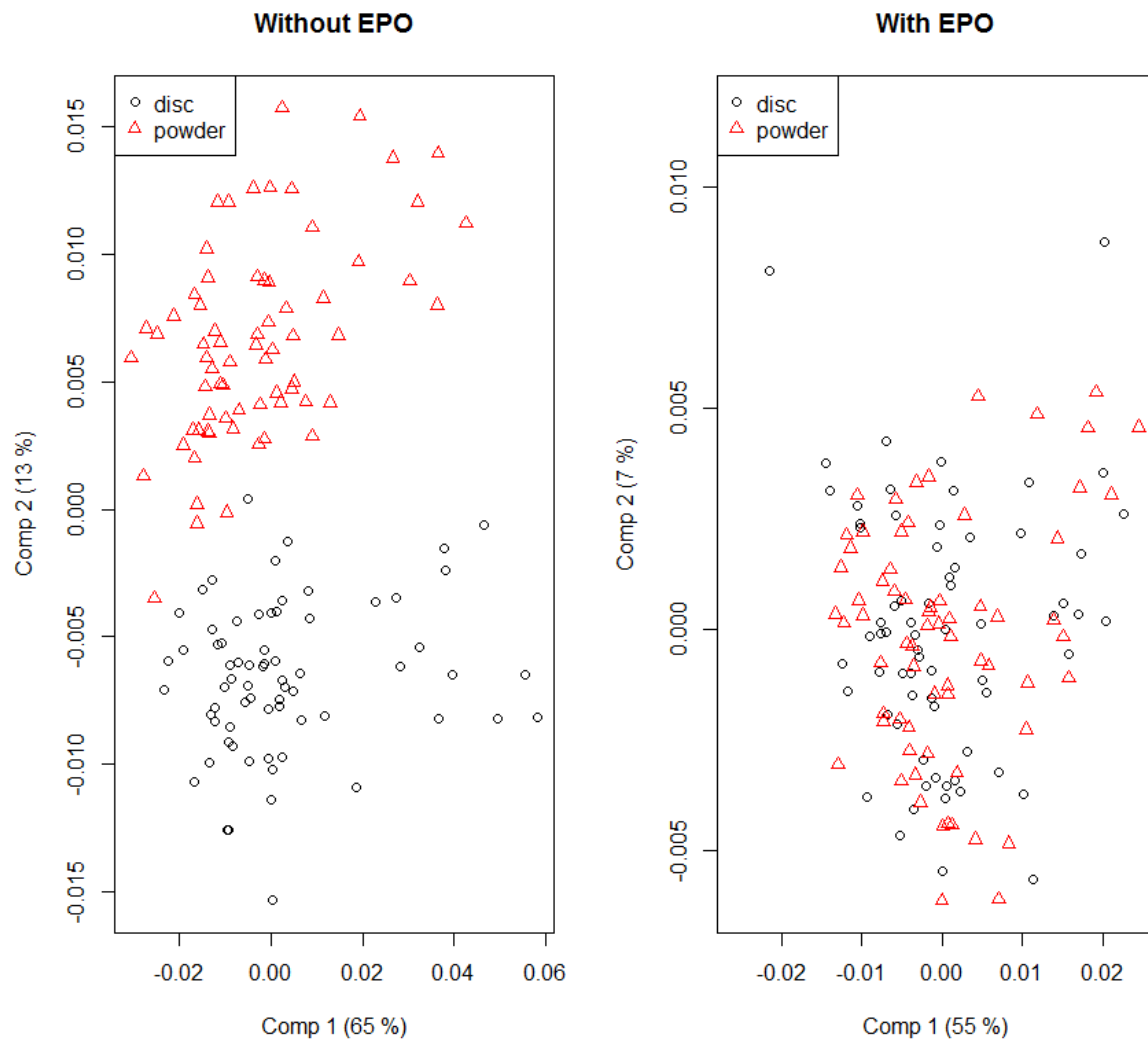
Model	Number of variables	Calibration			Validation	
		$R^2_{CV}$	$RMSE_{CV}$	Ncomp	$R^2_P$	$RMSE_P$
sMC	173	0.87	1.27	8	0.92	0.99
All	1282	0.82	1.48	7	0.90	1.06

### 3.3.5 Inhomogeneous distribution of extractives

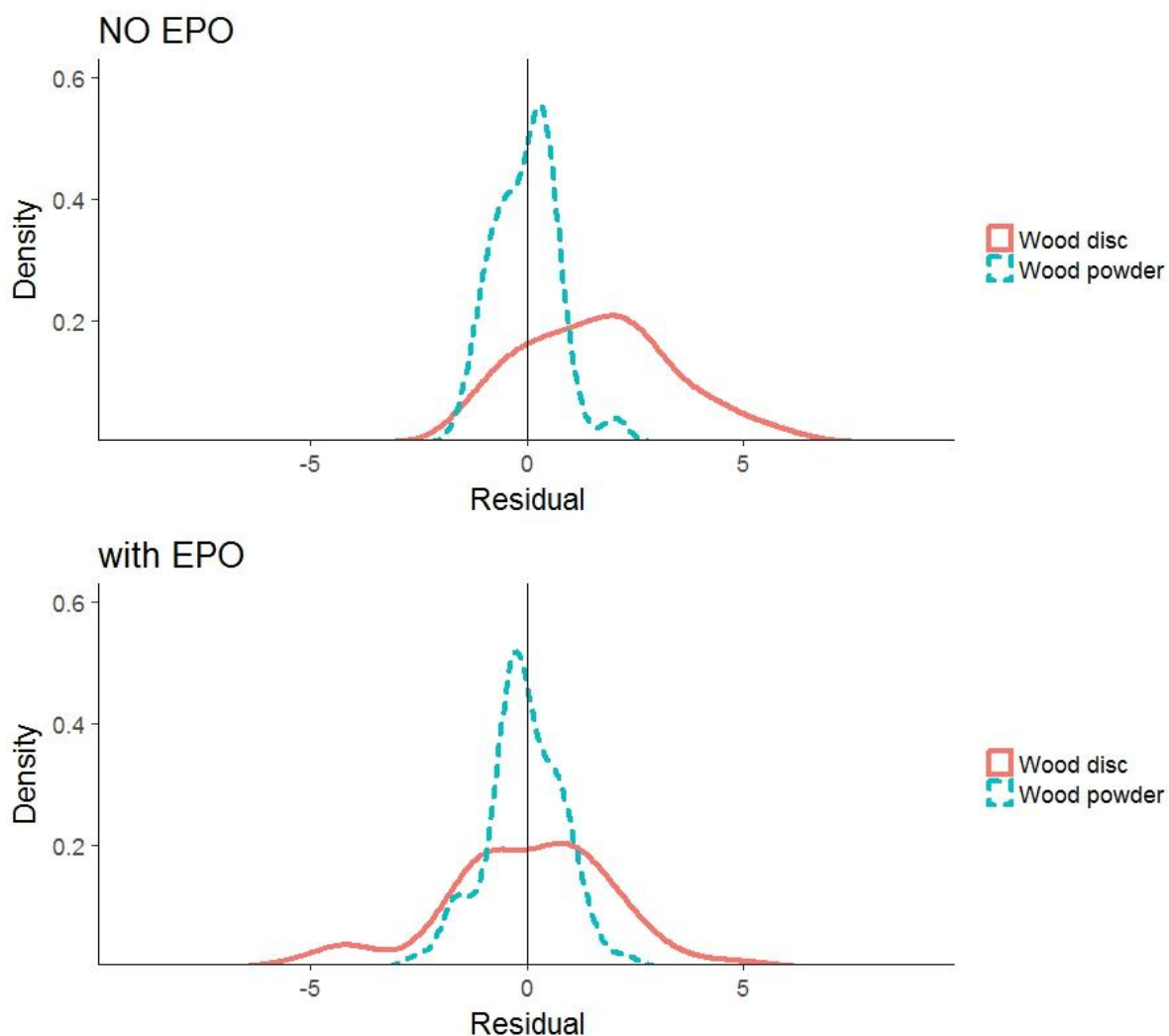
Extractives are not homogeneously distributed within a stem, increasing radially as well as varying locally (Gominho et al., 2007; Hillis, 1987; Morais and Pereira, 2012; Scheffer, 1966). Wood is milled and the EC averaged for the sample when determining the EC by solvent extraction for calibration. In contrast, EC is only averaged for the sampled area when acquiring spectra from solid wood, which in this study was  $\sim 2 \text{ mm}^2$ , the size of the fibre optic probe. Local EC variations are then captured by taking multiple spectra from the wood surface. The mismatch between extraction (milled wood) and NIR (solid wood) contributes to the RMSE of models built from solid wood NIR spectra. To investigate this effect, NIR spectra were obtained from the milled air-dry wood samples used for ethanol extraction. Milling can affect the spectra that need to be removed by EPO. Before calculating the EPO transformation between NIR spectra from discs and powder, both the disc and the powder spectra were subjected to the EPO-MC transformation to remove the influence of MC, and the disc spectra were also subjected to the EPO-grain transformation to remove the influence of sample form. The score plots of the PLS calibration model with and without EPO-milling transformation ( $c = 2$ ,  $h = 3$ ) showed that the wood disc and wood powder NIR spectra were clearly discriminated without EPO-milling transformation (Figure 3-6). This indicated that the predicted EC will be biased when using a model built from NIR spectra of another sample form. However, the distinction was removed after EPO-milling transformation (Figure 3-7). A bias of  $\sim 2\%$  was found between powder and solid wood

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spectra for the simple model when predicting EC, which was removed after the application of EPO-milling (Figure 3-6).



**Figure 3-6** Score plot of PLS calibration models for EC in *E. bosistoana* heartwood based on the EPO data set using the NIR spectra in air-dry condition from the transverse face of discs and milled samples; without EPO transformation (left) and with EPO transformation (right).



**Figure 3-7 Density plot of predicted EC (%) residuals of *E. bosistoana* heartwood before (top) and after (bottom) EPO-transformation for the EPO data set.**

The calibration data set was used to build PLS models with and without EPO-milling (EPO-PLS) after EPO-MC transformation. The contribution of inhomogeneous distribution of extractives in solid wood to the error of the EC predictions was assessed by comparing the RMSE of models based on milled and solid wood after removing the effect of sample form. As expected, the  $RMSE_{CV}$  was with 0.87% smaller for the wood powder than for the wood disc model, which had a  $RMSE_{CV}$  of 1.27% (Table 3-7). Therefore, we concluded that the local variation of extractive content in the solid wood samples contributed an error of ~0.5% to the calibration model. The effect of local variation in EC contributing to the error of the model can also be seen from the wider distribution of residuals for the disc-based model (Figure 3-6). The model based on powdered samples will be more accurate for



future predictions of EC in solid samples as it is possible to apply EPO transformation to remove the influence of sample form. Assessing solid samples has the advantage of minimal sample preparation, enabling to increase sampling of trees for example in a breeding program.

**Table 3-7 Performance of PLS regression models based on solid (discs) and milled wood for EC of *E. bosistoana* heartwood using EC model data set after EPO transformation, variable selection and spectra pre-processing (SNV+1<sup>st</sup> derivative).  $R^2_{CV}$ ,  $R^2_P$ : coefficient of determination - cross-validation (CV) and coefficient of determination of prediction when the model is applied to validation data set (P);  $RMSE_{CV}$ ,  $RMSE_P$ : root mean square error - cross-validation (CV) and root mean-square error of prediction when the model is applied to validation data set (P); Ncomp: number of components in the PLS model.**

	Number of variables	Calibration			Validation	
		$R^2_{CV}$	$RMSE_{CV}$	Ncomp	$R^2_P$	$RMSE_P$
Powder	88	0.94	0.87	7	0.96	0.69
EPO-PLS						
Disc	173	0.87	1.27	8	0.92	0.99
EPO-PLS						

### 3.3.6 Validation of the models on stem cores

The accuracy of the EPO-PLS models based on wood powder and wood disc spectra were tested using 36 wood cores of *E. bosistoana*. Figure 3-8 shows the measured EC against the predicted EC, both by the EPO-PLS wood powder and the EPO-PLS wood disc models. Both models reliably predicted the EC with a similar correlation. Considering the range of extractive content in the *E. bosistoana* populations the method reliably separated trees with high EC from those with low EC.

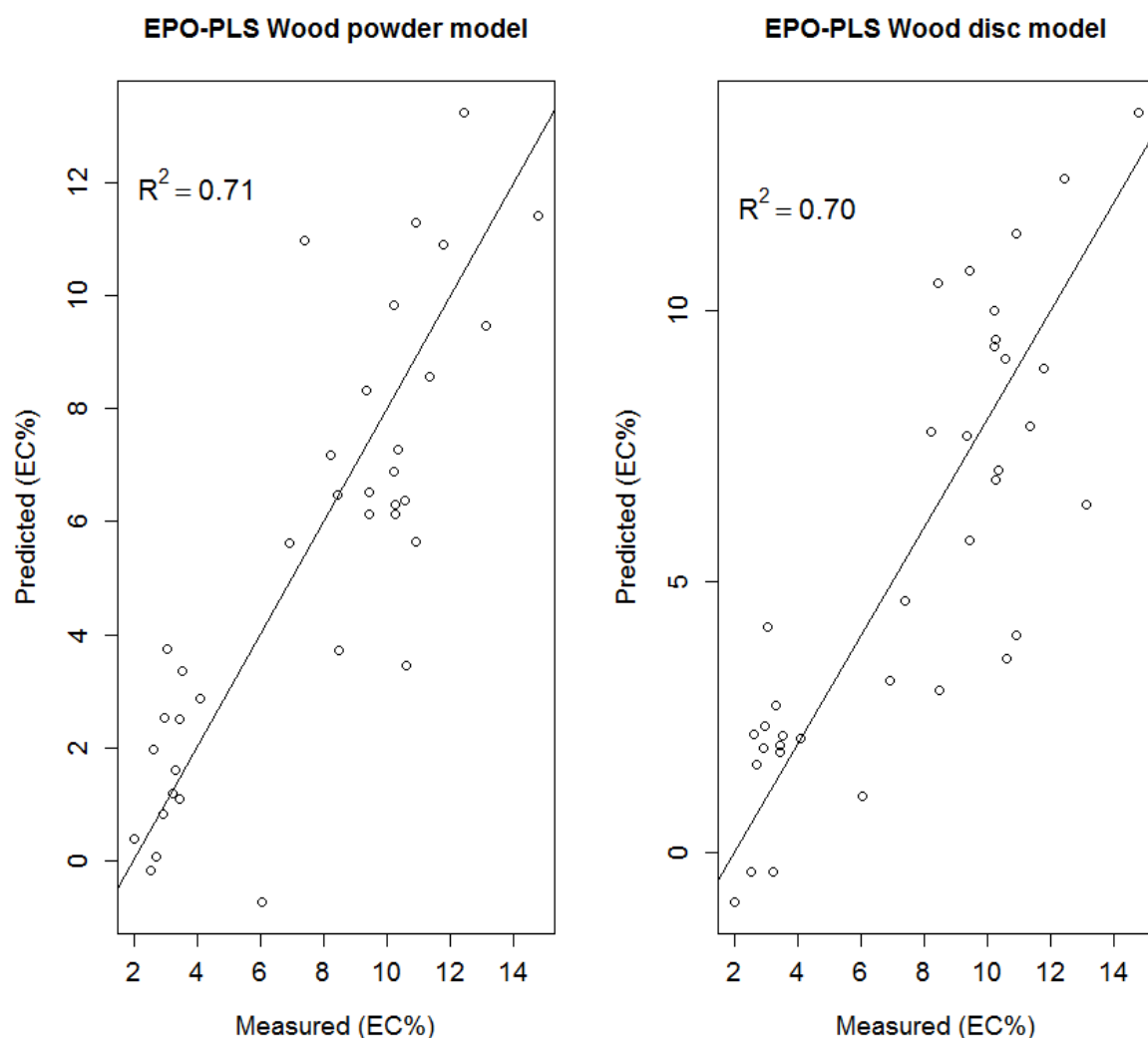


Figure 3-8 Measured and predicted EC (%) in heartwood of 36 *E. bosistoana* cores (Core validation data set) using EPO-PLS models based on wood powder and discs.

### 3.4 Conclusions

EC in heartwood of *E. bosistoana* could be quickly (seconds) and reliably predicted with NIR spectroscopy from solid wood samples with an RMSE of less than 1%. This was possible by removing effects of moisture, grain and sample form from NIR spectra by EPO transformation and selection of significant variables using the sMC algorithm. Removing the need to mill wood samples and control their MC for analysis provides a fast and non-destructive method enabling the cost effective analysis of the chemical composition of wood.

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The developed method will be used to include EC into the *E. bosistoana* breeding program. The method also allows to investigate the variable spatial distribution of extractives within stems. In future, especially if combined with NIR cameras, the here developed data processing approach will facilitate applications in tree breeding programs for screening for heartwood quality as well as in wood processing operations to grade timber for natural durability due to being faster and non-destructive.

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## **Chapter 4 Application of Near Infrared spectroscopy to heartwood of three durable eucalypts for quantification of extractives and species identification**

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### **4.1 Introduction**

Plantations are a productive and sustainable forest system for the production of fibre and timber. Commercial forest plantations are based on a select number timber species, which, although producing quality wood, are not durable. Durability of wood describes the resistance of timber to biodegradation (Scheffer and Morell, 1998). Many timber applications, in particular applications in moist environments such as out-doors or in-ground, require elevated levels of durability (ASTM, 2005; EN350-1, 1994; NZS3602, 2003). While there are technological processes to improve the durability of non-durable wood these either result in toxic waste (preservatives) (Eaton and Hale, 1993; Townsend and Solo-Gabriele, 2006), are expensive (chemical modification) or have a limited effect (thermal modification) (Hill, 2006). Alternatively, some timber species have naturally durable heartwood of excellent durability (Bootle, 2005; Scheffer and Morell, 1998) without added toxic chemicals or

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manufacturing costs. Unfortunately high natural durability is uncommon among tree species and those which are utilized are mostly rare and/or harvested unsustainably from tropical forests (Nellemann, 2012). Examples are kwila/merbau (*Intsia spp.*), mahogany (*Swietenia spp.*) or rosewood (*Dalbergia spp.*), which command the highest prices in the international timber trade (ITTO 2017; UNODC, 2016).

With the exception of teak (*Tectona grandis*) (Kaosa-ard et al., 1998; Thulasidas and Bailleres, 2017), which is restricted to tropical climates, no significant effort has been made to grow naturally ground-durable timber in plantations. The New Zealand Dryland Forests Initiative (NZDFI) has identified the high-value market demand for sustainable grown naturally durable timber. The NZDFI was established in 2008 as a collaborative tree-breeding and forestry research project to improve eucalypts that produce high quality naturally ground-durable hardwood required for New Zealand's agricultural, transport and energy sectors as well as specialty wood products for export to international markets (Altaner et al., 2017; Apiolaza et al., 2009; Walker, 2011). As eucalypts are quite site specific such durable eucalyptus plantations are most productive when grown as mixed species stands, matching the right species to the right site, an emerging concept in plantation forestry (Nichols et al., 2006; West, 2014). Target species for the NZDFI are *Eucalyptus bosistoana*, *E. globoidea*, and *E. argophloia*.

A prerequisite for a successful durable hardwood industry is to ensure that the produced timber meets the high wood quality requirements. This can be achieved through a breeding program (Jayawickrama, 2001; Raymond, 2002; Whiteman et al., 1996; Zobel and Jett, 1995). However, wood properties are rarely included in tree-breeding programs as they are typically resource consuming to measure.

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Near infrared (NIR) spectroscopy is a fast, non-destructive technique able to assess material properties which is used in industrial process for quality control (Osborne et al., 1993). Numerous studies have investigated its potential in the wood processing industry (Tsuchikawa and Kobori, 2015; Tsuchikawa and Schwanninger, 2013). A large proportion of the work uses NIR spectroscopy to quickly predict conventionally time- and cost-intensive to measure wood properties in tree-breeding programs. Common to most of these studies is that the required calibrations consider only samples from a single species sometimes restricted to one site and therefore have a proof-of-concept character. As a consequence, these calibrations are seldom used in a wider context and applied on a commercial scale. Calibrations based on samples from several species and sites have the potential to be applicable in a wider context. Downes et al. (2009) used nine distinct sample sets, which represented over 40 mainly eucalypt species spanning multiple sites to build a multi-species NIR calibration model for predicting Kraft pulp yield. The accuracy of the predictions with the multi-species model equalled that of the single species models and the larger calibration data covered a wider range of wood chemistries. Multi-species (He and Hu, 2013; Zhou et al., 2015a; Zhou et al., 2015b) and multi-site (Gebreselassie et al., 2017) NIR calibration for chemical wood traits aimed at servicing breeding selections have also been reported.

In the context of establishing a quality resource of naturally durable eucalypts, natural durability is the key wood property. The difficulty of conventionally measuring natural durability is prohibitive for its inclusion in a tree-breeding program (Harju and Venäläinen, 2006). As the natural durability of wood is primarily determined by extractives in the heartwood, NIR spectroscopy has the potential as a rapid, cost-effective and non-destructive technique to assess this trait in a breeding program. Previous studies have successfully used NIR to predict extractive content (Bush et al., 2011; Poke et al., 2005; Taylor et al., 2011) or

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decay resistance (Gierlinger et al., 2004) of wood. However, these were restricted to individual species.

Furthermore, in a commercial mixed species forest plantation it is important to separate timber from different species as these have typically different wood properties (Bootle, 2005) and therefore processing parameters and application products. NIR was used successfully to discriminate wood species for commercial or conservation purposes (Adedipe et al., 2008; Lazarescu et al., 2017; Schimleck et al., 1996; Shou et al., 2014). NIR was further used to locate the geographic origin of timber of a single species (Nisgoski et al., 2016) or for the identification of hybrids (Espinoza et al., 2012).

The objective of this work was a) to develop a cross-species NIR calibration for EC to reduce calibration needs in the durable eucalypt breeding program and b) to test if NIRS can be used to distinguish between species to ensure product quality in processing logs from mixed forest plantations.

## **4.2 Materials and methods**

### **4.2.1 Sampling**

Wood discs were collected from a variety of locations in New Zealand, including 36 samples from 6-year old *E. argophloia* grown in Marlborough, 71 samples from 7-year old *E. bosistoana* grown in Marlborough and 109 samples from 5- to 80-year old *E. globoides* grown at several sites along east coast regions of the upper South Island and lower North Island. The samples were taken either from an established stand of single or mixed species or research breeding trials set up by New Zealand Dryland Forests Initiative (NZDFI). In total, 216 samples were collected. Subsequently, the heartwood in each wood disc was isolated by drilling into the disc on the transverse face with a 12-mm drill. The drill ‘dust’ was collected

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and milled into a finer powder with a Wiley mill fitted with a 20 mesh (0.85 mm) screen. Then wood powder samples were placed in a climate-controlled room (25 °C, 60% RH) to obtain a stable moisture content (MC ~9%) before further processing.

#### 4.2.2 NIR spectroscopy

NIR spectra of wood powder were taken using a fibre-optics probe (Model N-500) attached to a NIR spectrometer (Tensor 37, BRUKER, Germany) at wavelengths from 9000 to 4000  $\text{cm}^{-1}$  at intervals of 4  $\text{cm}^{-1}$  and recorded with the OPUS\_7.5.18 software. Thirty-two scans were averaged for each sample. The wood powder was placed in an aluminium container fitting the fibre-optics probe. Each sample was measured three times, and each set of spectra was averaged.

#### 4.2.3 Extraction

After NIR spectra were collected, wood powders were oven dried at 60 °C. Subsequently samples were placed into a desiccator and allowed to cool to room temperature. Approximately 5-8 g of wood powder was weighed exactly into a stainless-steel cell and extracted twice with an Accelerated Solvent Extractor (ASE) (Thermo Scientific) at 70 °C for 15 min with ethanol. The extract solution was collected and transferred into a dry aluminium foil cup of known mass. The ethanol was left to evaporate overnight and then the extracts were oven dried at 105 °C. The mass of each extract was measured after cooling to room temperature in a desiccator and the extractives content (EC) was calculated on a dry mass wood basis.

#### 4.2.4 Multivariate analysis

The R software package (version 3.1.2) was used for data analysis (R Core Team, 2017). In total, 180 samples, including 30 *E. argophloia*, 55 *E. bosistoana* and 95 *E. globoidea*, were selected using Kennard-Stone sampling as calibration data sets, and the remaining 36 samples

(6 *E. argophloia*, 16 *E. bosistoana* and 14 *E. globoidea*) were used as validation data sets (Table 4-1). For this work all spectra were converted into their 2<sup>nd</sup> derivatives using Savitzky-Golay smoothing with a window size of 15 data points. The prospectr package (Stevens and Ramirez-Lopez, 2014) was used for NIR spectra manipulation and Kennard-Stone sampling. The plsVarSel package (Mehmood et al., 2012) implementing the significant Multivariate Correlation (sMC) algorithm was used to identify the variables in the NIR spectra most significantly correlated to EC in the PLSR models as well as for variable selection with a significance level of  $\alpha = 0.05$  (Tran et al., 2014). The packages caret (Kuhn, 2008) and pls (Mevik et al., 2015) were used for partial least squares-discriminant analysis (PLS-DA) and partial least square (PLSR) regression. PLSR training used leave-one-out cross-validation to select the optimal number of components for the calibration models.

**Table 4-1 Summary statistics of ethanol soluble extractive content (EC) in heartwood of *E. argophloia*, *E. bosistoana* and *E. globoidea* for the used datasets. CV: Coefficient of variation; SD: standard deviation;  $n_a$ ,  $n_b$ ,  $n_g$ : number of *E. argophloia*, *E. bosistoana* and *E. globoidea* samples in the dataset, respectively;  $n$ : number of samples in the combined data set.**

Data set	Species	Number of samples	Min	Max	Mean	SD	CV
Calibration data	<i>E. argophloia</i>	$n_a = 30$	4.64	18.85	10.91	3.05	0.28
	<i>E. bosistoana</i>	$n_b = 55$	0.96	14.67	5.30	2.75	0.52
	<i>E. globoidea</i>	$n_g = 95$	0.34	13.51	3.84	3.01	0.78
	Mixed species	$n = 180$	0.34	18.85	5.47	3.86	0.71
Validation data	<i>E. argophloia</i>	$n_a = 6$	6.48	15.31	10.41	3.22	0.31
	<i>E. bosistoana</i>	$n_b = 16$	2.33	13.36	3.96	2.58	0.65
	<i>E. globoidea</i>	$n_g = 14$	0.84	12.94	4.64	4.50	0.97
	Mixed species	$n = 36$	0.84	15.31	5.30	4.16	0.79

### 4.3 Results and discussion

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#### 4.3.1 Extractives content

Summary statistics for the calibration and validation data sets are shown in Table 4-1. In average, the *E. argophloia* heartwood samples contained twice as much extractives (~10%) as the *E. bosistoana* (~4.5%) or *E. globoidea* (~4%) samples. The coefficient of variation was highest for *E. globoidea* followed by *E. bosistoana* and *E. argophloia* in both data sets. A wide range of EC (<1% to >18%) was present in the data sets, which benefited the development of a stable and robust prediction model.

#### 4.3.2 Influence of species on EC prediction

The PLSR algorithm calculates components successively representing variation in the data and their correlation to the response variable (Wold, 2004). More of the variation in the data set will be explained by an increasing number of components. However, at some stage the model of the response variable will become less general. To avoid overfitting, i.e. ensuring that the developed model is applicable to data not included in the calibration dataset, the ideal number of components needs to be chosen. Figure 4-1 shows PLSR model characteristics for predicting EC in heartwood of three eucalyptus species using data for individual and mixed species data sets. The optimal number of components for the data sets ranged between 2 and 4. The achievable  $RMSE_{CV}$  was highest for the *E. argophloia* model (1.5%) followed by the *E. bosistoana* (1.1%) and *E. globoidea* (0.9%) models. The number of samples as well as the range of EC can influence the quality of a PLSR model (Borer et al., 1998). While the range of EC was similar for the three species, with *E. argophloia* sitting at a higher level, the *E. argophloia* model was based on significantly fewer samples ( $n = 30$ ) than those for *E. bosistoana* ( $n = 55$ ) and *E. globoidea* ( $n = 95$ ) (Table 4-1). This indicated that approximately 100 samples were needed to obtain a robust PLSR model for prediction of EC in heartwood of durable eucalypts from NIR spectra, a number comparable to that commonly advised for

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multivariate calibrations of NIR spectra (Chin and Newsted, 1999; Garson, 2016; Minasny et al., 2011).

Although little is known on the exact chemical composition of the heartwood extractives for the three eucalypt species, the majority of the compounds should have a similar chemical structure due to their relatedness (Benouadah et al., 2018; Brooker, 2000; Hillis, 1991; Hillis et al., 1974; Rudman, 1964). Therefore, combining the data from the three species might be beneficial to predict EC from NIR spectra as both, the number of samples as well as the range of EC, could be increased. The mixed species calibration model yielded a good result with stable performance for the validation data set ( $RMSE_P = 1.07\%$ ) (Table 4-2). The quality of the predictions was similar to the result reported by (He and Hu, 2013) who used NIR to predict EC in different wood species and obtained a  $R^2$  value of 0.95 but used double the number of components.



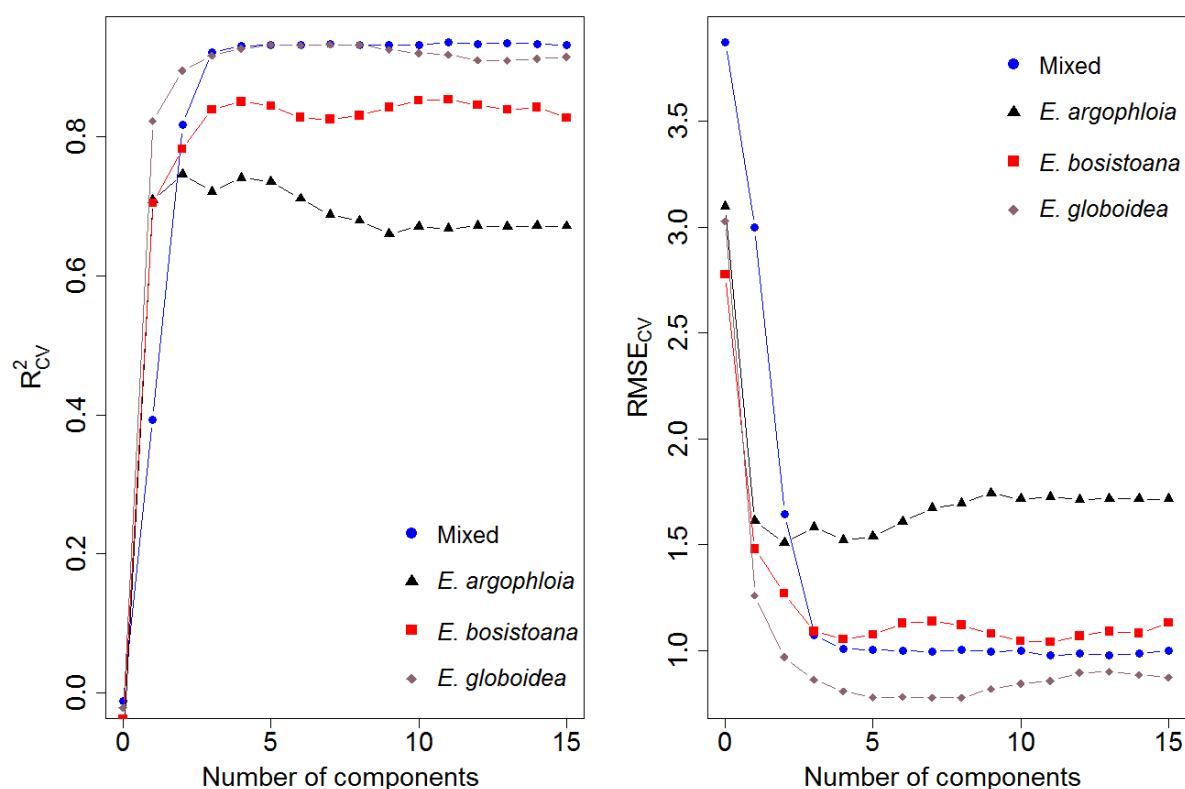


Figure 4-1  $R^2_{CV}$  (left) and  $RMSE_{CV}$  (right) of PLSR models predicting EC in heartwood of three *Eucalyptus* species from NIR spectra based on single species and mixed species data sets

Table 4-2 Characteristics of optimal PLSR models for EC in heartwood of three *Eucalyptus* species from 2<sup>nd</sup> derivative NIR spectra for the calibration and validation data sets.  $R^2_{CV}$ : coefficient of determination of cross-validation;  $R^2_p$ : coefficient of determination of prediction when the model was applied to the validation data set;  $RMSE_{CV}$ : root-mean-square error of cross-validation;  $RMSE_p$ : root mean-square error of prediction when the model was applied to validation data set.

PLSR Model	Calibration			Validation	
	$R^2_{CV}$	$RMSE_{CV}$	Number of components	$R^2_p$	$RMSE_p$
Mixed species	0.93	0.99	3	0.92	1.07
<i>E. argophloia</i>	0.74	1.52	2	0.77	1.41
<i>E. bosistoana</i>	0.85	1.05	4	0.76	1.23
<i>E. globoides</i>	0.92	0.86	3	0.93	1.17

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Figure 4-2 showed the correlation between predicted and measured EC of the calibration and validation data for the three eucalyptus species using the model developed from the combined data. No obvious bias between the species was present. Combining the data sets improved the precision of the predicted EC for all three species compared to PLSR models based on the individual species data sets, as the range of residuals decreased (Figure 4-3). In particular, the range of residuals decreased for the *E. argophloia* dataset, indicating the benefit of a larger sample set.

Future predictions of EC in heartwood of durable eucalyptus species from NIR spectra can be based on mixed species calibrations. This will reduce cost and labour of adding other species, as fewer samples need to be analysed and added to the existing data set that would be necessary for a single species calibration.

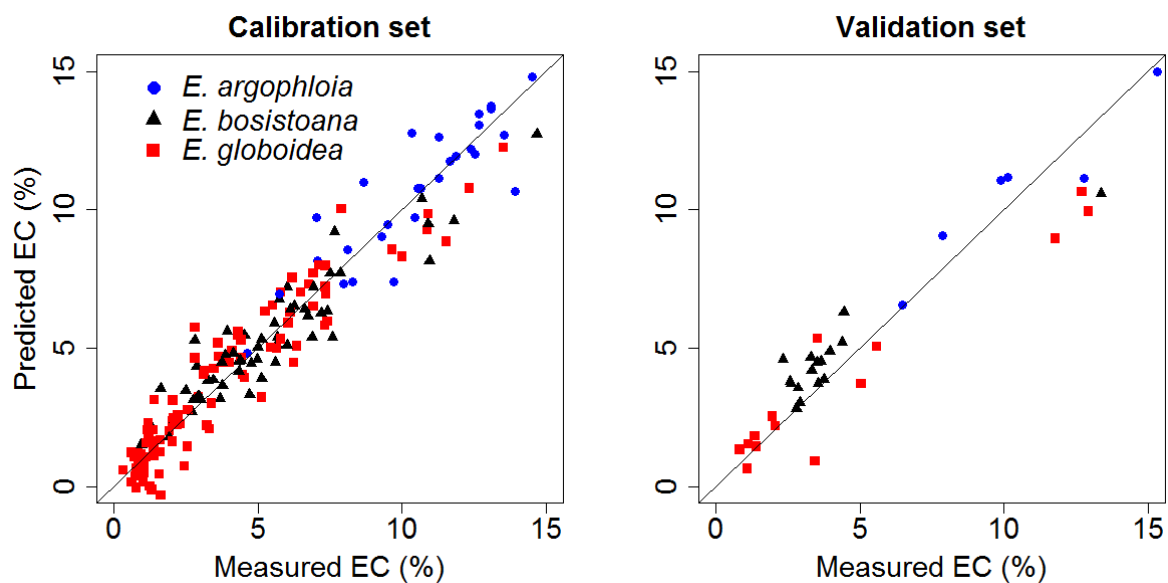
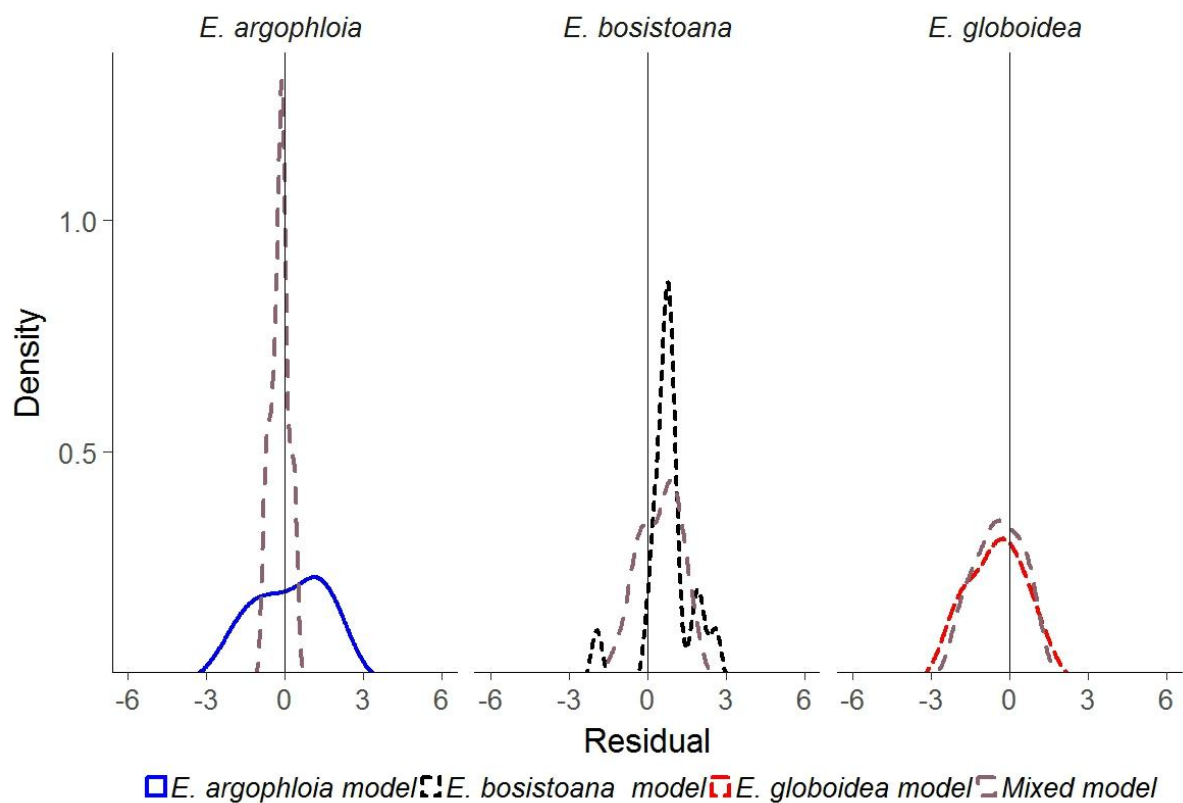


Figure 4-2 Measured and predicted EC in heartwood of three eucalyptus species in both, the calibration (left) and validation (right) data sets based on 2<sup>nd</sup> derivatives of NIR spectra



**Figure 4-3** Density plot of predicted EC (%) residuals of *E. argophloia*, *E. bosistoana* and *E. globoides* samples from validation data sets using mixed species and their single species model.

### 4.3.3 Variable selection

A variable selection algorithm (sMC) (Tran et al., 2014) was applied to a) improve the quality of the PLSR models by removing unimportant variables and b) reveal NIR signals which correlated most to the EC in heartwood of the three eucalyptus species.

Characteristics of PLSR models to predict EC of heartwood including variable selection (sMC) for individual species and the mixed species data are shown in Table 4-3. The sMC algorithm reduced the number of selected variables for all models from the original 1296 data points. Focusing the models on those selected variables improved their precision by more than 10% to a RMSEP of 0.9%.

**Table 4-3** PLSR model characteristics to predict EC of heartwood in three *Eucalyptus* species from NIR spectra using single and mixed species data sets after variable selection (sMC).  $R^2_{CV}$ : coefficient of determination of cross-validation;  $R^2_P$ : coefficient of determination of prediction when the model was applied to the validation data set; RMSE<sub>CV</sub>: root-mean-square error of cross-validation; RMSEP: root mean-square error of prediction when the model was applied to validation data set.

PLSR Model	Variables selected	Calibration			Validation	
	by sMC	$R^2_{CV}$	RMSE <sub>CV</sub>	Number of components	$R^2_P$	RMSEP
Mixed species	268	0.95	0.90	4	0.95	0.90
<i>E. argophloia</i>	70	0.90	0.96	2	0.89	0.97
<i>E. bosistoana</i>	110	0.93	0.72	4	0.95	0.55
<i>E. globoides</i>	325	0.94	0.71	4	0.97	0.78

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The average 2<sup>nd</sup> derivative NIR spectra of the three species were similar, indicating the similarity in chemical composition of the heartwood of the three species (Figure 4-4). This was to be expected due to the general similarity of wood cell walls (Pettersen, 1984) and the heartwood extractives (Hillis, 1991). In general, the sMC algorithm identified the same regions centred around 6865, 6017, 5265 and 4656 cm<sup>-1</sup> in each model (Figure 4-4). However, the relative importance of these differed between the datasets.

Assigning NIR signals to chemical structures is difficult due to coupling and overlap (Siesler et al., 2002). Wood and wood component related NIR signal assignments have been summarized (Schwanninger et al., 2011). Bands around 6000 cm<sup>-1</sup> have been associated with the 1<sup>st</sup> overtone of C-H stretching vibrations of methyl groups or acetyl groups. This signal has also been reported to correlate to the band at 4656 cm<sup>-1</sup> in eucalyptus, which was associated with combinations of aromatic C-H and C-C stretching vibrations as well as the combination of C-H and C=O stretching vibrations of acetyl groups (Michell and Schimleck, 1996). The 1<sup>st</sup> overtones of phenolic O-H groups were reported to vibrate around 6874 cm<sup>-1</sup> (Fackler and Schwanninger, 2010). A signal at 5245 cm<sup>-1</sup> has been associated with the 2<sup>nd</sup> overtone of C=O stretching vibrations in hemicelluloses. However, this region is overlapped by a strong water signal and therefore less likely to be reliably quantified in moist wood. The assignment of these peaks to aromatic, phenolic, methyl and carboxyl groups agrees with what is known about the compounds present in extractives of eucalyptus heartwood including gallic-, ellagic-, methylellagic- acid, catechin and resveratrol (Barbosa et al., 2005; Hillis, 1991; Rudman, 1964). To a lesser degree, these groups are also present in cell wall polymers lignin and hemicelluloses.

The signals at ~6017 and 4656 cm<sup>-1</sup>, associated with heartwood extractives in eucalyptus (Michell and Schimleck, 1996) had the largest contribution in all models except for the *E. bosistoana* (Figure 4-4). The strong contribution from the 6865 cm<sup>-1</sup> band, associated with

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phenolic OH groups, in the *E. bosistoana* model could be an indication that heartwood compounds of this species are richer in this functional group than the other two species. No signals were found in the region from 9000 to 7000  $\text{cm}^{-1}$ , which strongly related to the extractive content in heartwood of the three eucalyptus species. This region was reported to be influenced by particle size and colour changes of wood (Schwanninger et al., 2011). The fact that similar regions were selected for all species gives confidence that a mixed species model to predict EC is feasible.

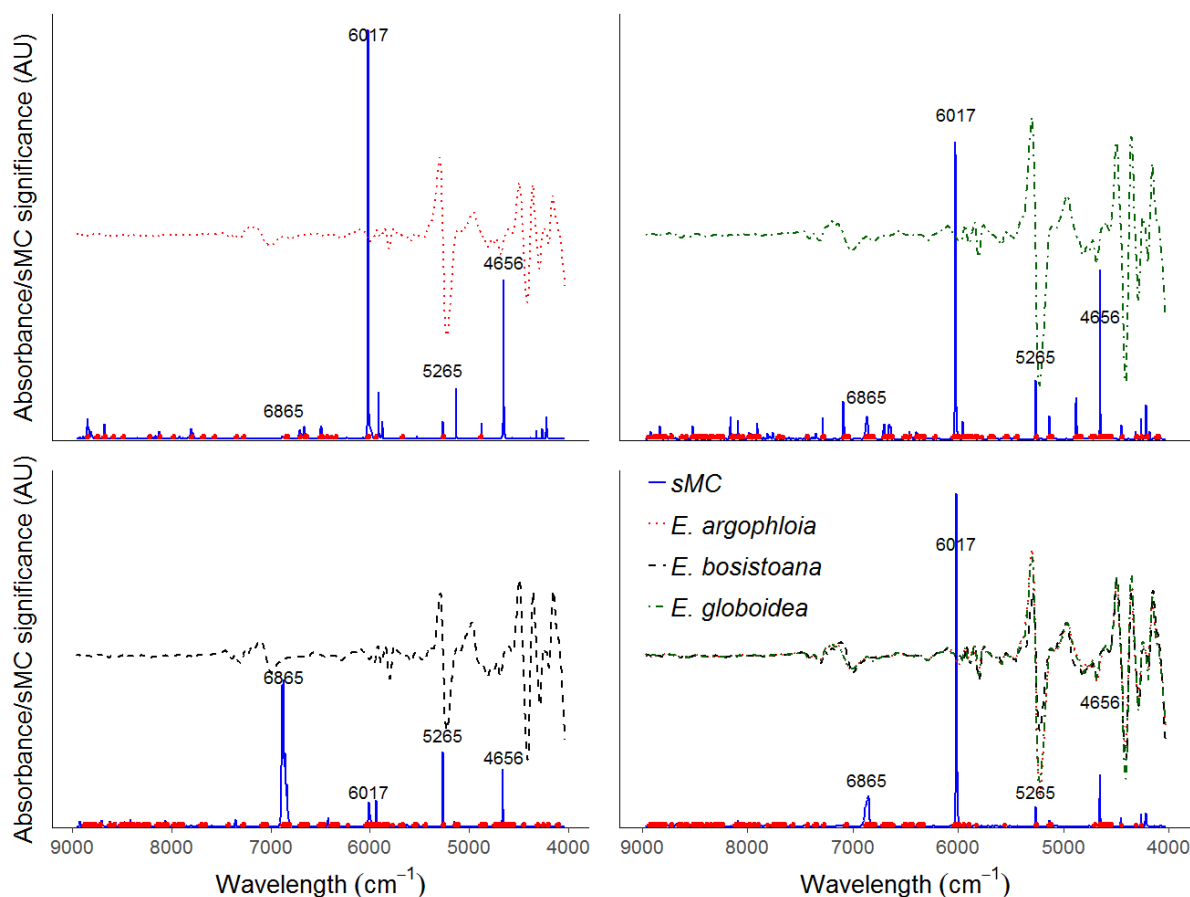


Figure 4-4 2<sup>nd</sup> derivative NIR spectra (dashed/dotted lines), explained EC variation calculated by the sMC algorithm (solid line) and selected variables by sMC (red points) (upper left: *E. argophloia* model, upper right: *E. bosistoana* model, bottom left: *E. globoidea* model, bottom right: mixed species model).

#### 4.3.4 Differentiating heartwood of three eucalyptus species

Apart from utilizing the similarity between the three eucalyptus species to create a robust NIR calibration for EC of their heartwood, the same data set can also be analyzed for the differences between the species. In a commercial situation where species of different quality (such as durability for *E. globoidea* (Class 2) and *E. bosistoana* (Class 1) (Bootle, 2005)) are mixed it is of interest to quickly identify them. PLS-DA was used to investigate if the three eucalyptus species can be differentiated from NIR spectra of their heartwood. *E. argophloia*, *E. bosistoana* and *E. globoidea* were well separated in the PLS-DA scores plot (Figure 4-5). Using three principal components, the PLS-DA model distinguished the heartwood of the three eucalyptus species with 100% accuracy of (Table 4-4). Similar results were reported for

pinus for which a PLS-DA model with less than 5 components based on 2<sup>nd</sup> derivative NIR spectra of wood could successfully identify four species (Hwang et al., 2016), or for *Tsuga heterophylla* and *Abies amabilis* two similar timbers of different wood quality (Lazarescu et al., 2017).

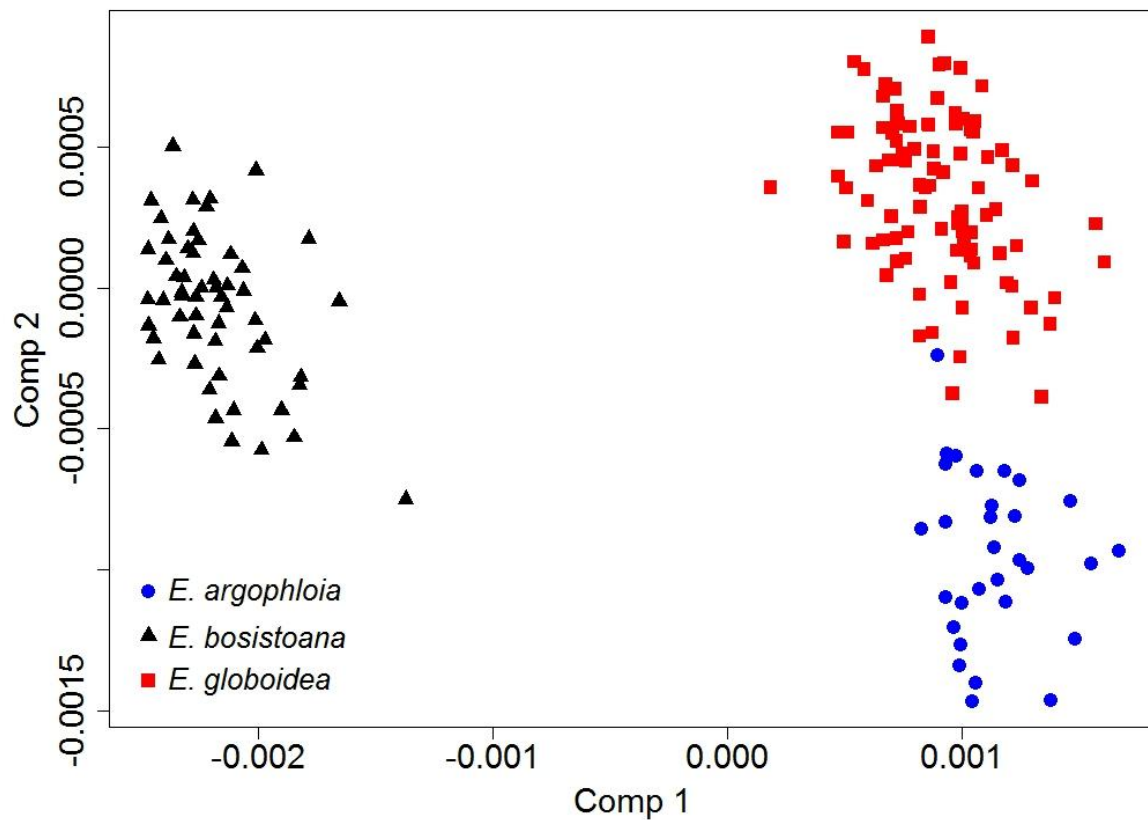


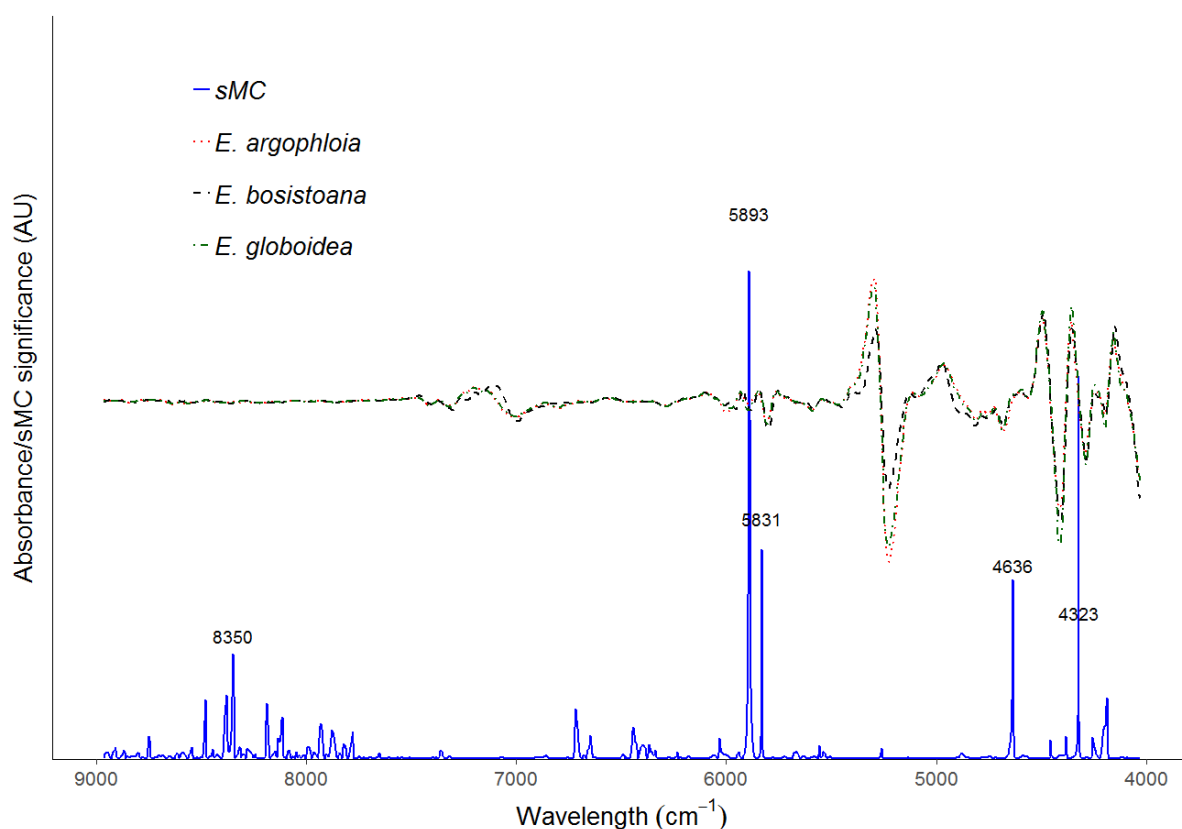
Figure 4-5 Scores plot of the 1<sup>st</sup> and 2<sup>nd</sup> principal components of the PLS-DA model using 2<sup>nd</sup> derivatives of NIR spectra to discriminate *E. argophloia*, *E. bosistoana* and *E. globoidea* heartwood



**Table 4-4 PLS-DA model characteristics to discriminate *E. argophloia*, *E. bosistoana* and *E. globoidea* heartwood from using 2<sup>nd</sup> derivatives of NIR spectra.  $n_a$ ,  $n_b$ ,  $n_g$ : number of *E. argophloia*, *E. bosistoana* and *E. globoidea* samples in the dataset, respectively.**

Data set	Number of components	Species	Number of samples	Accuracy (%)
Calibration set	3	<i>E. argophloia</i>	$n_a = 30$	100%
		<i>E. bosistoana</i>	$n_b = 55$	100%
		<i>E. globoidea</i>	$n_g = 95$	100%
Validation set	3	<i>E. argophloia</i>	$n_a = 6$	100%
		<i>E. bosistoana</i>	$n_b = 16$	100%
		<i>E. globoidea</i>	$n_g = 14$	100%

The difference in the NIR spectra of heartwood between the three eucalyptus species can be caused by chemical as well as anatomical differences. To identify the signals in the NIR spectra, which contributed most strongly to the PLS-DA model, the sMC algorithm was applied (Figure 4-6). The five most important regions selected by the sMC algorithm were centred at 8350, 5893, 5831, 4636 and 4323  $\text{cm}^{-1}$ . The signal around 8350  $\text{cm}^{-1}$  and 5893  $\text{cm}^{-1}$  were close to bands previously assigned to the 2<sup>nd</sup> and 1<sup>st</sup> overtones of  $\text{CH}_3$ -groups and C-H stretching of lignin, respectively (Schwanninger et al., 2011). For the 5831, 4636 and 4323  $\text{cm}^{-1}$  bands no reasonable assignment related to wood or wood components was published (Schwanninger et al., 2011).



**Figure 4-6** Frequencies selected by sMC for discriminating *E. argophloia*, *E. bosistoana* and *E. globoidea* heartwood from 2<sup>nd</sup> derivatives of NIR spectra.

## 4.4 Conclusion

NIR is a promising and reliable method to verify the quality of natural durability eucalyptus wood and to classify similar eucalyptus species. Models showed a high prediction and classification performance and were based on few PLSR components. The multi-species EC calibration will be used in the NZDFI breeding program. In particular, this study showed that

- a mixed species model to predict EC performed a better than the single species models for all three species
- most improvement in the performance was gained for small sample sets, implying that another eucalypt species, potentially introduced into the NZDFI program to create hybrids, could be included in the model with a relatively small sample set, removing a main barrier of this technology

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- the most important signals for predicting EC located at 6865, 6017, 5265 and 4656  $\text{cm}^{-1}$  were shared between the three eucalypt species and were assigned to chemical groups common to compounds present eucalypt heartwood extractives
  - the three eucalyptus species could be successfully separated with NIR spectroscopy, based on bands not related to those identified important to predict the EC.

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## Chapter 5 **Distribution of extractives in trees**

### **5.1 Introduction**

Wood is composed of lignin, cellulose and hemicelluloses as well as various extractives. Variation of extractives in heartwood can have far-reaching effects on the quality and value of wood, in particular for products like furniture or outdoor applications. For optimal utilisation of wood, the chemical, aesthetical and mechanical properties have to be well-defined. However, only the mechanical properties are commonly considered by the solid timber industry. Wood extractives influence physical and mechanical properties of timber, for example, extractives can reduce swelling and shrinking of wood by lowering the equilibrium moisture content (EMC) (Fengel and Wegener, 1983; Popper et al., 2007). More importantly, the extractives in heartwood are the primary cause for increased natural durability (Hawley et al., 1924).

The relationships between extractive content (EC), colour and natural durability are complex. Heartwood colour is mainly as a result of the accumulated extractives, which can differ in their chemical composition within a species. Gierlinger et al. (2004) found that chemical components in the extractives determined colour in larch wood and that the colour of heartwood had a significant relationship with EC and natural durability, i.e. natural durability may be estimated from the colour of heartwood. However, for example in oak, the variation of wood colour was found to have no correlation with the EC in the inner heartwood but a strong correlation in the outer heartwood (Klumpers et al., 1993).

Studies have shown that the colour of heartwood has significant variation which is controlled by genetics and environment both between- and within-trees. These relationships are species specific. Bradbury et al. (2011) examined the variation of colour in Australian blackwood heartwood for 16 open-pollinated families with CIELab colour space measurements on 12-

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mm diameter stem cores after sanding. The results showed that the base colour is primarily controlled by genetics and nurse crops but affected little by rainfall. Therefore, the silviculture as well as genetic selection should be considered to obtain stability of heartwood colour. On the other hand, Rink (2007) reported that environmental factors were the main influences on the heartwood colour of black walnut.

Heartwood is formed in the transition zone, located between sapwood and heartwood, where physiological changes like removal of reserve materials like starch and the deposition of extractives occurs (Hillis, 1987; Taylor et al., 2002). As the wood in this zone is gradually transitioning from sapwood to heartwood, its durability and EC will not match that of heartwood. The transition zone was reported as ~1.5 cm in 15 year-old *Tectona grandis* trees (Hillis, 1987) and ~1 cm in 6 year-old *Eucalyptus bosistoana* young trees (Mishra et al., 2018). Consequently, assessment of heartwood needs to treat the outer ~1-1.5 cm as a transition zone.

Extractives, as well as colour and decay resistance, is not evenly distributed within the heartwood of a stem and can be highly variable (Zabel and Morrell, 2012). The EC typically decreases from the outer edge of the heartwood to the centre (pith), as well as with height in a stem (Rudman, 1964; Sherrard and Kurth, 1933). In *Thuja plicata*, this phenomenon was associated with juvenile trees (DeBell et al., 1999). Mosedale et al. (1996) found a logarithmic decline of EC from the heartwood boundary towards the pith in oak species. What is also mirrored by natural durability (AS5604, 2005). Consistent with this, the resistance of the outer heartwood was reported to decrease significantly from the lower to the upper trunk (Scheffer and Morrell, 1998).

Additional to these typical patterns within a stem, local variation of EC can also be found in heartwood. These can be regular, forming concentric ring patterns as in *Microberlinia*

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*brazzavillensis* (zebrawood) or irregular like in *Dacrydium cupressinum* (rimu) (Kohl, 2012; Wheeler, 2011). A homogeneous distribution of extractives within the wood should benefit the natural durability of timber.

Heartwood, extractives and natural durability of roots have been less studied. Heartwood formation was reported in roots of spruce and larch trees (Böttcher and Liese, 1975). In root heartwood of *Picea abies* high amounts of lignans were reported (Ekman, 1979). *Picea sitchensis* extractives of roots, for which it was not specified if they contained heartwood, were different from those found in heartwood, sapwood and knots of stems (Caron et al., 2013). However, these studies were based on roots sampled from only two trees. Polyphenols with oxidising properties were also reported from *P. abies* fine roots, which were most likely free of heartwood (Richter et al., 2007). Platt et al. (1965) reported that sapwood of roots from coniferous trees contained higher amounts of reserve carbohydrates and nitrogen than stem sapwood, which resulted in lower decay resistance.

Little is known about the EC distribution in root and stem heartwood of *E. bosistoana*. For early genetic selection for high EC it is essential to have information on the variation of EC within a stem to inform sampling position within a stem in terms of a) height and b) radial position. To facilitate early selection, samples should be taken where heartwood forms earliest in the stem, which is potentially at the root-stem interface. This chapter describes the radial and axial EC distribution in root and stem heartwood of *E. bosistoana* and *E. globoidea* trees.

## **5.2 Method and materials**

### **5.2.1 Materials**

Samples included (Table 5-1) one disc cut from the base of a ~40 year-old *E. bosistoana* tree grown in Marlborough, New Zealand, roots and tree stems collected in 2016 from two *E.*

*bosistoana* trees and one 7 year-old *E. globoidea* tree at the Craven's Rd (41°26'S, 173°56'E). Roots and stems were sampled from different trees. In addition, 1130 *E. bosistoana* stem cores collected in 2016 from three breeding trials (Lawson's (41°43'S, 174°02'E) - subdivided into an East facing and a North facing slope) and Craven's Rd (41°26'S, 173°56'E)), which were planted by NZDFI on 2009 were used (Chapter 3). All sites were located in Marlborough, New Zealand.

All the roots smaller than 50 mm diameter were removed from the primary root (Figure 5-1 and 5-2). The primary roots were cut into discs at 10 cm intervals resulting in five discs each. From the stems, discs (~50 cm long) were collected every 1 m. Only disc that contained heartwood were analysed for EC distribution. Core (transverse face) and disc surfaces were sanded with P100 grid sandpaper and placed into an air controlled room (25 °C, 60% RH) for at least one month to obtain a stable moisture content (~9%) before collecting NIR spectra.

**Table 5-1 List of samples used to study the distribution of EC within *E. bosistoana* and *E. globoidea* trees.**

Species	Type	Number	Age
<i>E. bosistoana</i>	core	1130	7
<i>E. bosistoana</i>	root	2	7
<i>E. globoidea</i>	root	1	7
<i>E. bosistoana</i>	stem	2	7
<i>E. globoidea</i>	stem	1	7
<i>E. bosistoana</i>	stem	1	~40



Figure 5-1 unpruned *E. bosistoana* root. Discs 1 to 5 are from the top of the root (above ground) to the bottom of the root (below ground); with only disc 1 coming from an above ground part of the tree.



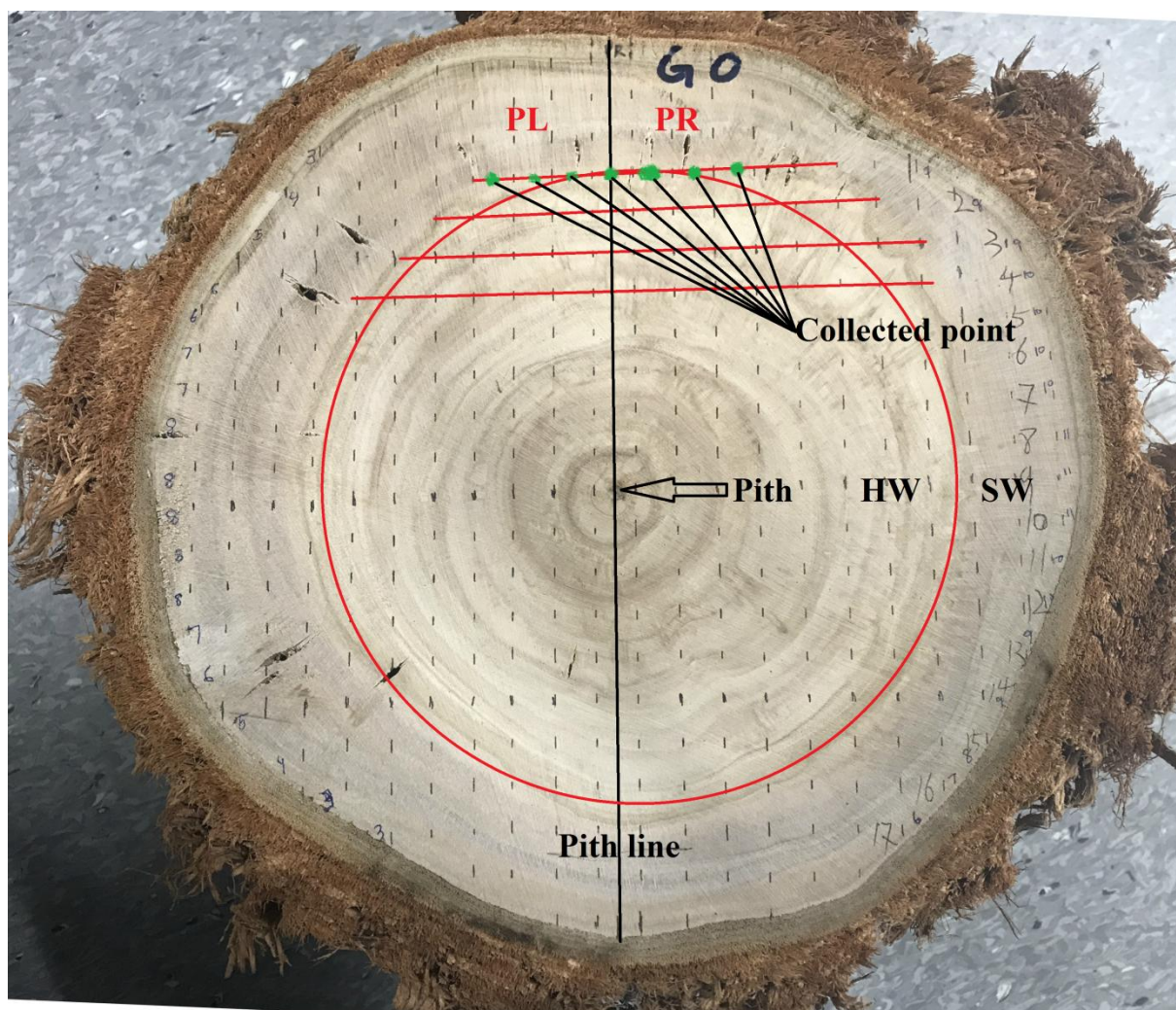
Figure 5-2 pruned *E. globoidea* root. Discs 1 to 5 are from the top of the root (above ground) to the bottom of the root (below ground); with only disc 1 coming from an above ground part of the tree.

### 5.2.2 Data collection and EC prediction

NIR spectra were collected on each point. Spectra were acquired between 9,000 to 4,000  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  intervals. 32 Scans were averaged for each spectrum. NIR spectra were collected



from each core sample from the sanded surface with a fibre optics probe (Model N-500, Bruker Optics, Germany) every 5 mm along the heartwood. Each disc was measured as displayed in Figure 5-3. Only discs that contained heartwood were analysed. Spectra were taken from the heartwood but not the sapwood area. It should be noted that the demarcation was not sharp and also blurred by the transition zone. The distance between the rows and between the measurements was 10 mm. PR side collection points start from the pith line. PL side start from 10 mm away from pith line.



**Figure 5-3 Map of NIR measurement points on discs, HW: heartwood. SW: sapwood. PL: pith left side. PR: pith right side. It can be seen that some measuring points fell into the transition zone. No spectra were taken from the sapwood.**

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### 5.2.3 Data analysis

EC was predicted for each spectrum using the EPO solid wood PLSR model developed in chapter 3. Briefly, R 3.1.2 (R Core Team, 2017) was used for data processing. The prospectr package (Stevens and Ramirez-Lopez, 2014) and the plsVarSel package (Mehmood et al., 2012) were used for NIR spectra pre-processing and variable selection. Each sample spectra was predicted using the pls package (Mevik et al., 2015). The plot3Drgl (Soetaert, 2016) and ggplot2 packages (Wickham, 2009) were used for 3D and 2D graphs of EC.

For radial EC profiles of discs the Cartesian x, y coordinates were converted into a polar r,  $\alpha$  coordinate system with the pith at the centre. The radius for each measurement point  $i$  could be calculated as:

$$\text{Radius}_i = \sqrt{x_i^2 + y_i^2}$$

This allowed averaging and modelling radial profiles in the discs. The relative position of a measurement in the heartwood was calculated by dividing the radius of each point by the maximum radius in each disc.

$$\text{Relative radial position}_{ij} = \frac{\text{Radius}_{ij}}{\text{Max}(\text{Radius}_j)} \times 100$$

where  $\text{Radius}_{ij}$  is the value  $i$  in disc  $j$ . This should normalise for differences in tree growth and highlight age effects. A linear regression model was used to test the effect of radial position on EC within cores. It can be symbolized with the following model equation:

$$\text{EC} = \mu + \text{site} + \text{site:radius} + e$$

where  $\mu$  is the overall intercept, site the deviation of site from the intercept, radius the distance from the pith, and site:radius the interaction of site and radius, and  $e$  the residuals (response unexplained by the model predictors). This model allows for a different intercept



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and slope for each site. An alternative version of this model used the proportion of the core instead of the actual radius.

Generalized additive models (GAMs) (Hastie and Tibshirani, 1990) was used to test effects on EC within trees stems and roots. The GAM is a generalization of linear models that adds the effect of smoothers expressed by:

$$EC = s(Radius) + s(Tree\ height)$$

where  $s$  is a smoothing function. When modelling roots  $s(Tree\ height)$  is replaced by  $s(Root\ depth)$ .

## 5.3 Results and discussion

### 5.3.1 Radial distribution of EC

#### 5.3.1.1 Radial distribution of EC in a ~40 year-old *E. bosistoana* stem

The distribution of EC in heartwood of ~40 year-old disc was plotted in Figure 5-4. EC in the heartwood appeared to decrease from the outer heartwood towards the pith, an observation reported previously for other tree species (Hillis, 1987), including *Eucalyptus* spp. (Wilkes, 1984). The PLSR model identified no heartwood extractives in the pith.

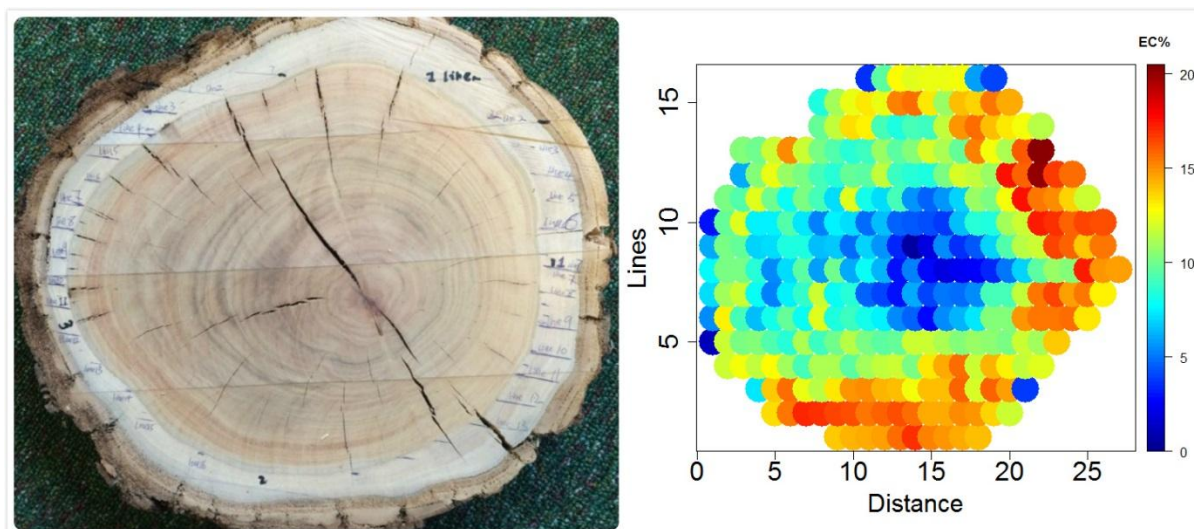


Figure 5-4 Distribution of EC in heartwood at the base of a ~ 40 year-old *E. bosistoana* stem predicted by NIR. The right side plot is only applied to the heartwood of the left side plot.

#### 5.3.1.2 Radial distribution of EC in 7 year-old *E. bosistoana* cores

The EC differed for wood types present along a stem radius (Figure 5-5). No heartwood extracts were predicted by the NIR model in sapwood (mean -0.1%). The EC in transition zone, defined as the extractive containing tissue within 10 mm of the sapwood, was with 4.1% lower than that of heartwood (9.2%). Although the zone close to sapwood contained heartwood extractives, it is likely that the transition to heartwood, i.e. accumulation of extractives, was not finalised. Similar results were found by Mishra et al. (2018), who reported that the transition zone in 6 year-old *E. bosistoana* trees was ~1 cm in radial width. Therefore, this zone should be excluded from assessing heartwood. The EC in the pith (3.5%) was lower than that of the heartwood, a result consistent with observations in larch (Gierlinger and Wimmer, 2007).

The EC in heartwood, excluding the transition zone, was plotted against the radial position differentiated by site for the 1130 tree cores of 7 year-old *E. bosistoana* (Figure 5-6). Significant variation between sites and site/radius were found (Table 5-2). The EC in different sites was variable, with the mean EC in the two Lawson's sites being similar and

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higher than at Craven's Rd. This was consistent with findings for trees from Craven's Rd planted in a different trial, which were also found to have low EC (Chapter 6).

The EC increased with radial position by 0.03%/mm in these young trees. The increase in EC in heartwood with radial position was consistent with the observation in the older tree disc (Figure 5-4) and literature reports (Hillis, 1987; Wilkes, 1984).

Modelling EC as dependent on the relative radial position within the heartwood should normalise the data for differences in tree growth rate, highlighting the effect of age. EC increased only little in respect of the relative radial heartwood position for the 7 year-old *E. bosistoana* trees (Table 5-2). Therefore, growth rate rather than age seemed to affect the radial increase in EC. The differences between trees appeared to be larger than within a radial profile (Figure 5-6), suggesting that young trees with high EC (i.e. likely to be of higher natural durability) can be found.

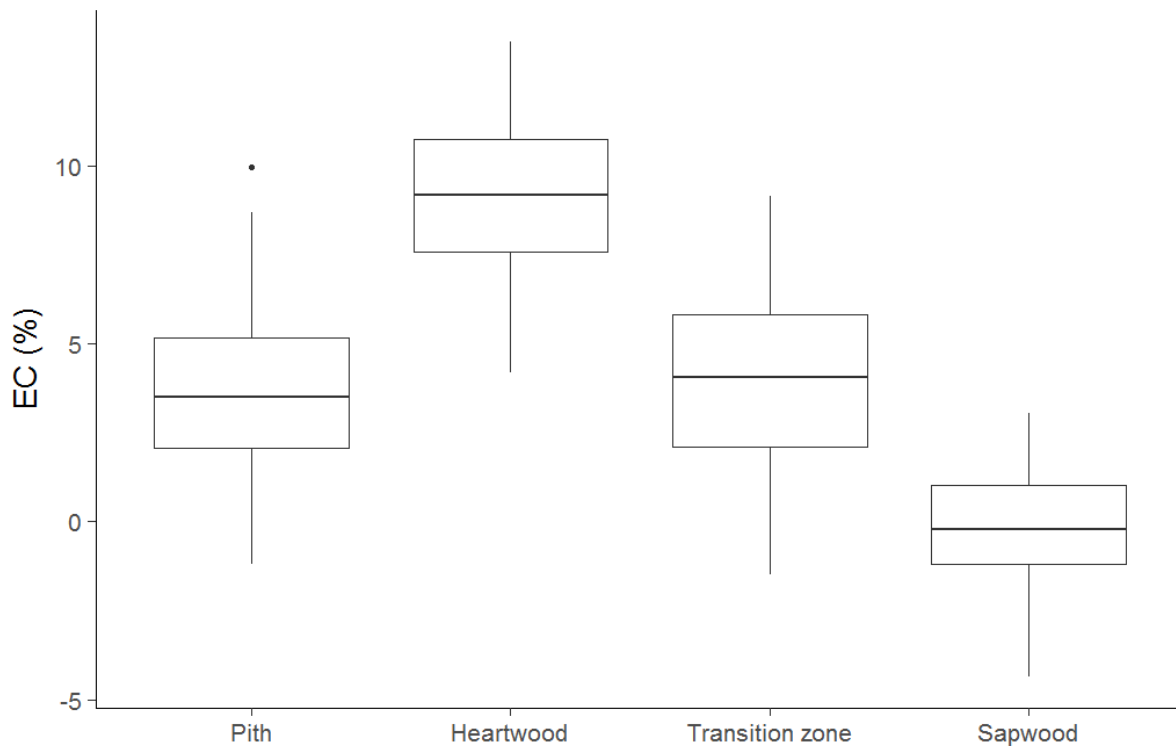


Figure 5-5 EC in sapwood, pith (within 5 mm from the pith), transition zone (10 mm of heartwood closest to the sapwood), and heartwood (rest of the heartwood) of 1130 7 year-old *E. bosistoana* tree cores.

Table 5-2 Linear regression model of heartwood EC dependent on radial position (excluding the transition zone) and site. Std. Error: standard error. Significance code: ‘\*\*\*’ 0.01, ‘\*\*\*\*’ 0.001.

		Estimate	Std. Error	t value	P-value
Radius	(Intercept)	2.46	0.10	23.97	<2E-16***
	Radius	0.03	0.01	3.18	1.47E-03**
	Lawson’s East	1.64	0.15	10.83	<2E-16***
	Lawson’s North	1.95	0.16	12.36	<2E-16***
	Lawson’s East: Radius	0.11	0.02	6.42	1.64E-10***
	Lawson’s North: Radius	0.08	0.01	5.56	3.04E-8***
Relative heartwood position	Intercept	2.29	0.13	9.86E+02	<2E-16***
	Relative position	7.39E-03	1.26E-03	1.70E+03	4.84E-09***
	Lawson’s East	1.81	0.19	1.01E+03	<2E-16***
	Lawson’s North	2.10	0.20	9.82E+02	<2E-16***

	Lawson's East: Relative position	6.55E-03	1.75E-03	1.69E+03	1.83E-4***
	Lawson's North : Relative position	5.26E-03	1.88E-03	1.69E+03	5.28E-3**

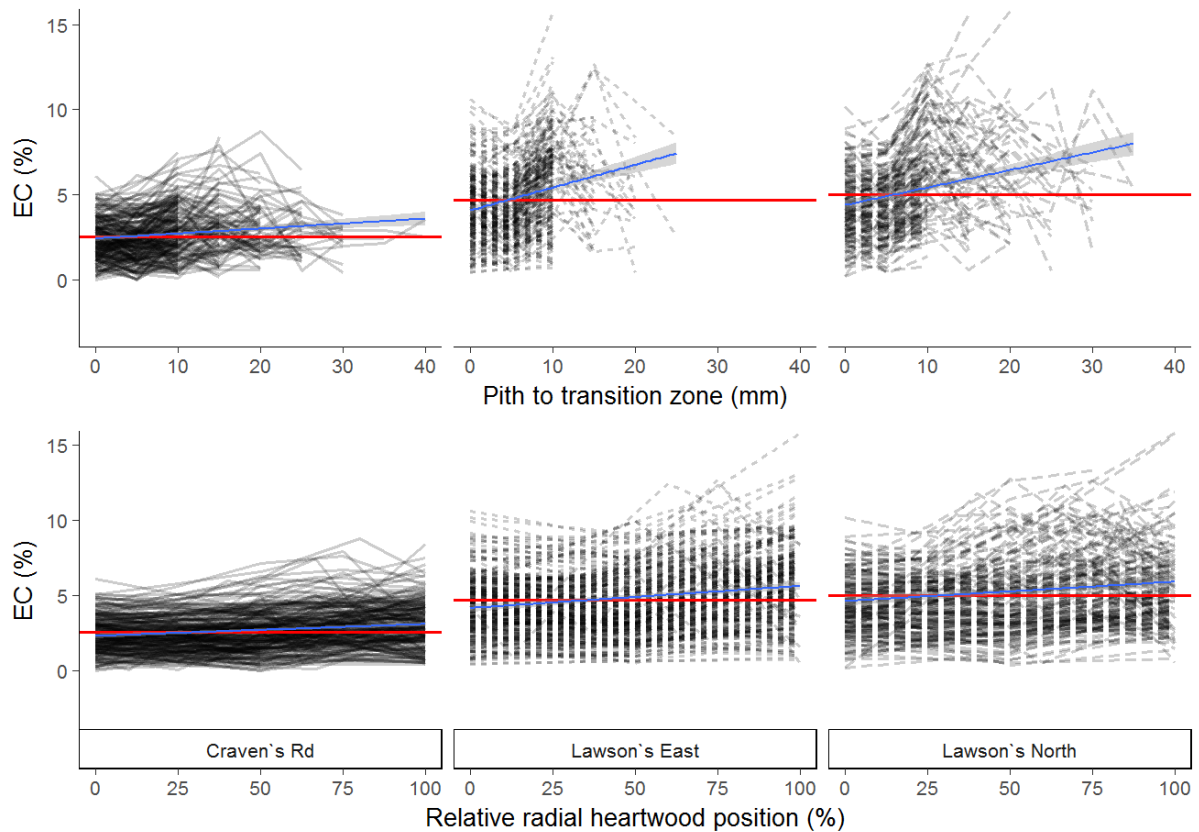


Figure 5-6 EC in heartwood of 1130 7 year-old *E. bosistoana* tree cores from three sites depending on radial position. Absolute radial position (top) and relative radial position in heartwood (bottom). Red line: median EC value of each site. Blue line: linear regression.

### 5.3.2 Longitudinal distribution of EC in heartwood of 7 year-old *E. bosistoana* and *E. globoidea* tree stems

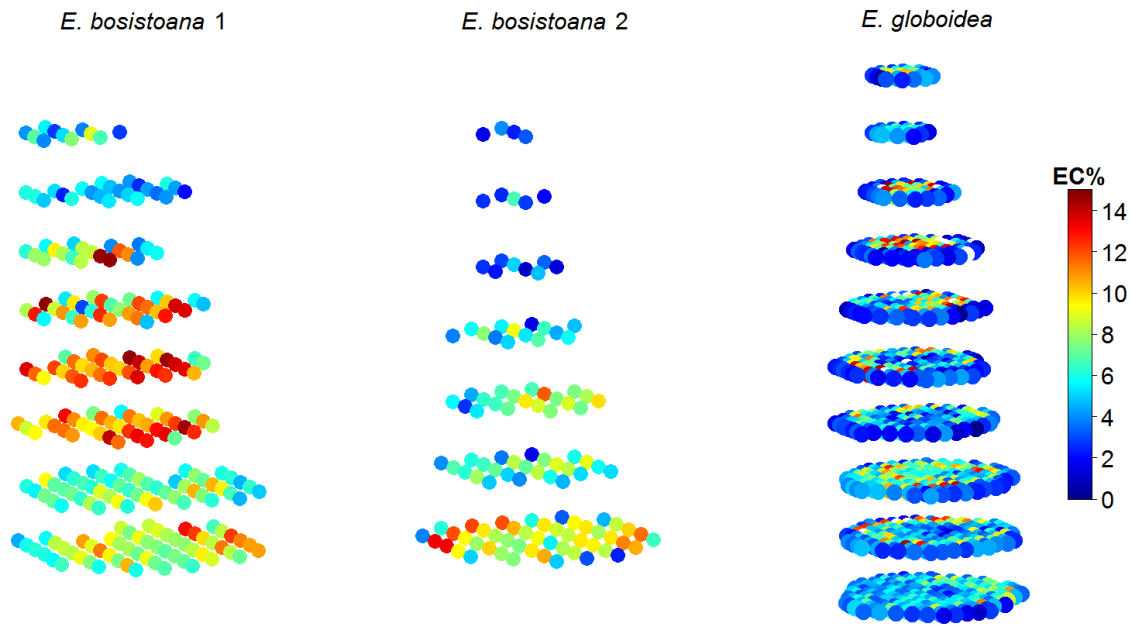


Figure 5-7 3D distribution maps of EC in heartwood of 3 tree stems. Stems were sampled at 1 m intervals.

Figure 5-7 shows the predicted EC distribution in heartwood of two *E. bosistoana* and one *E. globoidea* stems in 1000 mm height intervals from bottom to top. With the taper of the trees the heartwood area decreased with height. Axial as well as local variation in EC appeared to be present.

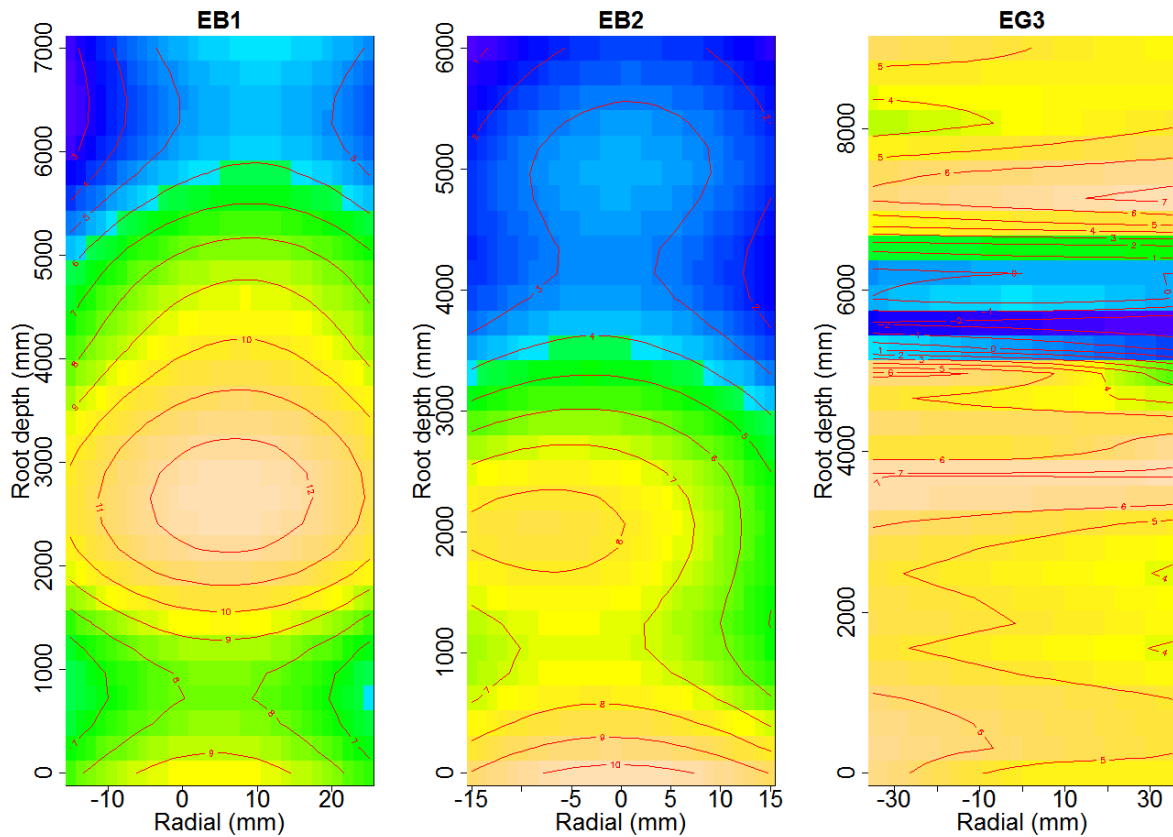
The averaged radial EC profiles in heartwood were calculated at each sampled tree height (Figure 5-8). For these, the transition zone, defined as the 20% of the extractive containing radius closest to the sapwood were removed. For the *E. globoidea* and the *E. bosistoana* 1 trees the EC was highest at intermediate stem height, i.e. a lower EC at the base and top of the tree stem. These were found to be highly significant (Table 5-3). The EC in the smaller *E. bosistoana* 2 stem decreased with stem height.

It should be noted that radial position had no significant influence on EC for the *E. bosistoana* 1 and *E. globoidea* trees and a negative correlation for the *E. bosistoana* 2 tree (Table 5-3). This might have been a result of the difficulty to remove the transition zone from

the analysis as the radii differ in length around the stem, i.e. the heartwood was not cylindrical.

**Table 5-3 Approximate significance of smooth terms of EC in heartwood (excluding the transition zone) for tree height and radius in 7 year-old two *E. bosistoana* and one *E. globoidea* stems. Std. Error: standard error. Significance code: ‘\*\*\*’ 0.01, ‘\*\*\*\*’ 0.001. EDF: estimated degrees of freedom, Ref. DF: estimated reference degrees of freedom.**

		EDF	Ref. DF	F value	P-value
<i>E. bosistoana</i> 1	Radius (mm)	1.61	2.01	5.56	4.22E-3**
	Tree height (mm)	1.98	2.00	58.19	< 2E-16****
<i>E. bosistoana</i> 2	Radius (mm)	1.65	2.06	5.91	3.17E-3**
	Tree height (mm)	1.00	1.00	81.22	4.22E-16****
<i>E. globoidea</i>	Radius (mm)	3.51	4.41	6.46	2.26E-05***
	Tree height (mm)	8.16	8.79	4.59	4.74E-06****



**Figure 5-8** Heat map of EC in the heartwood of three tree stems depended on radial position and height. EB1: *E. bosistoana* tree 1, EB2: *E. bosistoana* tree 2, EG3: *E. globoidea* tree. The value on the contour line is the EC. The 0 in the x axis radial (mm) means the pith. The decrease and increase from the pith mean the right and left side from the pith.

### 5.3.3 EC radial distribution and longitudinal distribution of 7 year-old *E. bosistoana* and *E. globoidea* tree roots

The distribution of heartwood EC in three primary roots of two *E. bosistoana* and one *E. globoidea* trees at 100 mm depth intervals from top to low depth was plotted in Figure 5-9. The highest EC was present below the ground where the primary root expanded and branched off into side roots. As a result the heartwood area was also largest at this depth.



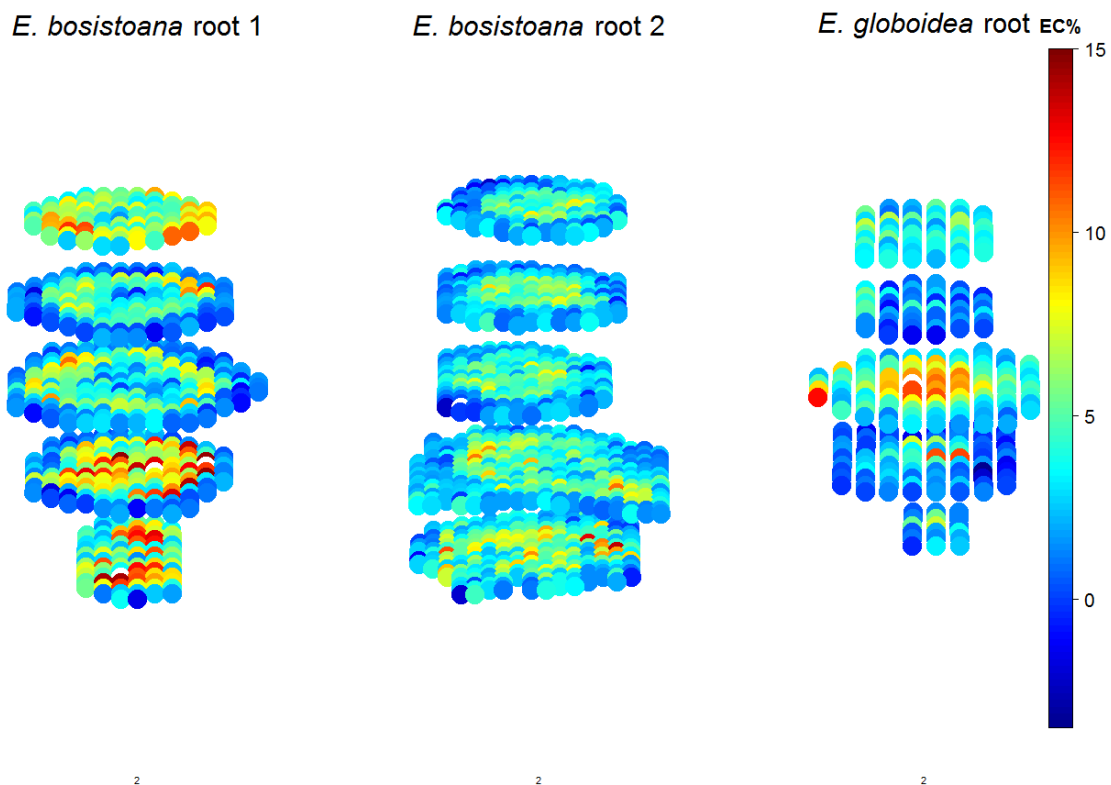


Figure 5-9 3D plot of EC in the heartwood of 3 tree roots. Roots were sampled at 100 mm intervals with the ground line represented by the 2<sup>nd</sup> disc from the top.

The radial and longitudinal EC profile in the roots for each assessed depth excluding the transition zone is shown in Figure 5-10. The ground line was at 400 mm. EC distribution in between the roots are difference. Radial local variation was present, radial position had significant effect on EC in the two *E. bosistoana* roots the *E. globoidea* root (Table 5-4). Within roots, root depths had a significant effect on EC in all trees. However, the highest EC was present at different depths.

The data showed, constant with literature (Böttcher and Liese, 1975) , that heartwood did not start at the bottom of the tree trunk but extended into the roots. Therefore sampling trees at the base of a stem for heartwood is feasible. However, the data does not allow to confirm the nature if the heartwood extractives in roots, which have been reported to differ from those of stems (Caron et al., 2013; Ekman, 1979).

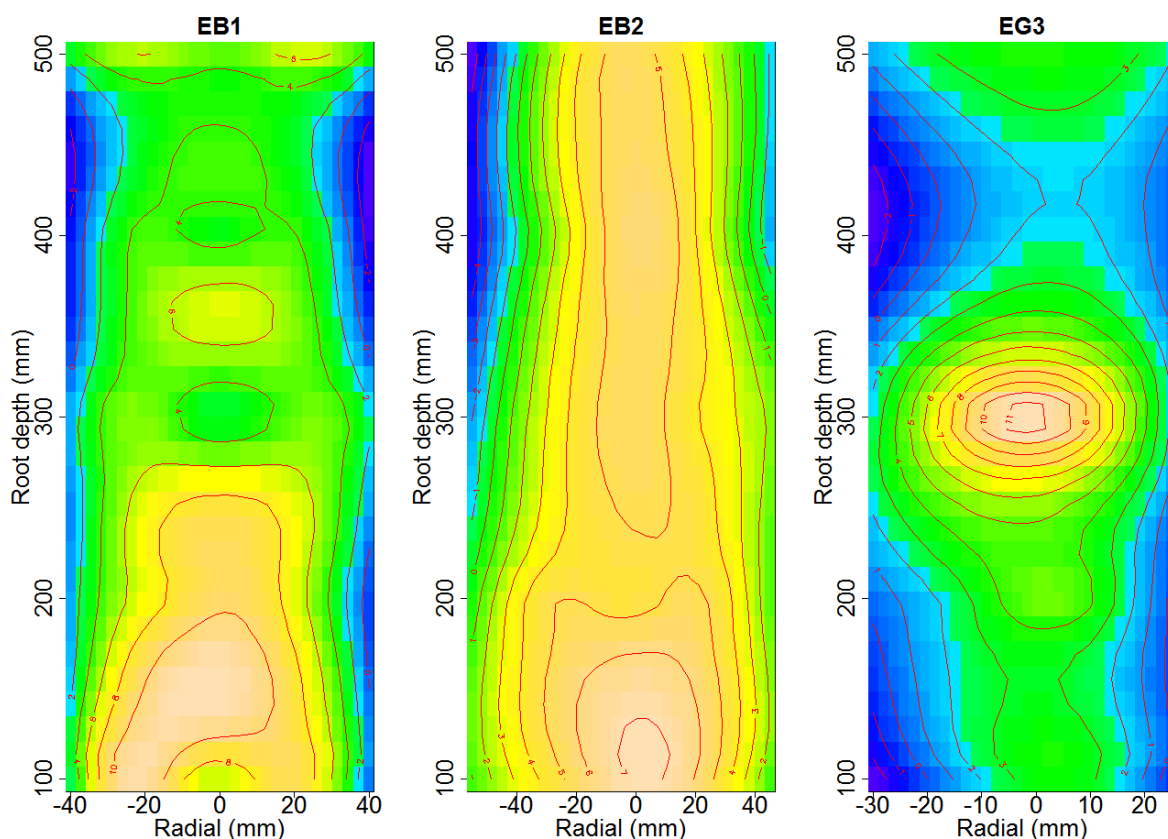


Figure 5-10 EC in heartwood of three tree roots depended on radial position and depth. Ground level at 400 mm. EB1: *E. bosistoana* root 1, EB2: *E. bosistoana* root 2, EG3: *E. globoidea* root. The value on the contour line is the EC. The 0 in the x lab radial (mm) means the pith. The decrease and increase from the pith mean the right and left side from the pith.

Table 5-4 Linear regression model of EC dependent on radial position for different root depths in roots of one *E. globoidea* and two *E. bosistoana* trees. Std. Error: standard error. Significance code: '\*\*\*' 0.01, '\*\*\*\*' 0.001.

		EDF	Ref. DF	F value	P-value
<i>E. bosistoana</i> 1	Radius (mm)	3.24	4.07	11.54	4.07E-09***
	Root depth (mm)	1.96	2.00	22.68	2.42E-10***
<i>E. bosistoana</i> 2	Radius (mm)	5.87	7.03	32.93	<2E-16***
	Root depth (mm)	1.67	1.89	55.74	<2E-16***
<i>E. globoidea</i>	Radius (mm)	2.58	3.24	14.37	4.49E-09***
	Root depth (mm)	1.98	2.00	23.05	4.09E-10***

## 5.4 Conclusion

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The EC varied in a typical pattern along a radius for 7 year-old *E. bosistoana*. Sapwood contained no while heartwood contained 9.2% extractives. The EC was with 3.5% lower in the transition zone, defined as the 10 mm of heartwood containing wood closed to the sapwood. This needs to be considered when assessing trees for EC, for examples in a breeding programme.

The extractive content in the heartwood, excluding the transition zone, increased from pith outwards. This increase seemed to be more governed by tree diameter than tree age. However, larger variation between trees than with a radial profile indicated that young trees with high extractive content can be found. This indicated that it is likely to produce naturally durable timber in short-rotation plantation forestry systems with these trees.

Heartwood extended into the roots. Axial gradients in EC seemed to be present in the roots as well as the stems of the investigated trees. The limited number of trees in this part of the study suggested that EC was highest at intermediate stem height and root depth.

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## Chapter 6 Genetic variation in heartwood properties and growth traits of *Eucalyptus bosistoana*

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Li, Y., Apiolaza, A. L., Altaner, C., Genetic variation in heartwood properties and growth traits of *Eucalyptus bosistoana*.

The manuscript presented here may differ from any published version due to peer-review and editorial processes.

### 6.1 Introduction

Most tree breeding programs for *Eucalyptus* species target improvements in tree growth and health, basic density and pulp yield, as plantations were primarily intended for pulp and paper products (Greaves and Borralho, 1996; Schimleck et al., 2004; Stackpole et al., 2010). Less work has been done on improving *Eucalyptus* solid-wood properties; in particular, work on

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heartwood properties is very scarce. As plantations and their breeding programs have evolved, for some species the range of traits assessed has increased to include natural durability (Bush et al., 2011).

Natural durability describes the resistance of wood to biological decay by fungi, insects or marine borers without any wood preservation treatments. The presence of wood extractives is the most important factor explaining the natural durability of wood (Hawley et al., 1924; Rudman, 1964). Extractives are deposited when the inner part of the sapwood is transformed into heartwood (Hillis, 1987; Taylor et al., 2002).

New Zealand vineyards preponderantly use softwood posts to support the vines. These posts are treated with copper/chrome/arsenate (CCA), as untreated radiata pine is not sufficiently durable to meet commercial standards (Bush et al., 2011). However, these wood preservation treatments result in costly and potentially harmful environmental problems (Townsend and Solo-Gabriele, 2006). These concerns have resulted in naturally durable wood being increasingly regarded as a desirable material for posts.

There is significant within-species variability on natural resistance to biodeterioration, which is partly controlled by genetics and possibly amenable to improvement through breeding (Hillis, 1987). Genetic variation in natural durability has been reported for various tree species, including *Larix sp.* (Gierlinger et al., 2004), *Thuja plicata*, *Chamaecyparis nootkatensis* (Taylor et al., 2006) or *Picea glauca* (Yu et al., 2003) as well as some eucalyptus species. Significant variation in fungal decay resistance has been reported in *Eucalyptus marginata* (Perry et al., 1985), *E. cladocalyx* (Bush, 2011) and *E. grandis* and *E. camaldulensis* × *botryoides* clones (Palanti et al., 2010). Bush (2011) also found that methanol extractive content in *E. cladocalyx* could be improved by breeding, a finding



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mirrored in *Pinus sylvestris* with reported heritability ranging from 0.5 to 0.7 for quantities of different extractives (Fries et al., 2000).

Apart from heartwood of good quality, it is also important that the trees produce large quantities of heartwood. Heartwood quantity has also been shown to be under genetic control. For example in *E. cladocalyx* (Bush, 2011) or *P. sylvestris* (Ericsson and Fries, 1999).

For genetic variability, the effect of environmental variables should also be considered. Lima et al. (2000) found significant clone  $\times$  site interaction for wood density of 26 *Eucalyptus* clones in four sites at eight-years-old. There is also evidence of plantation site influencing heartwood decay resistance in *Tectona grandis* (Moya and Berrocal, 2010). Gierlinger et al. (2004) reported that the heartwood/sapwood ratio and the axial development of heartwood within *E. globulus* show high levels of Genotype  $\times$  Environment (G  $\times$  E) interaction. Moraes et al. (2002) suggested that site differences may indirectly influence the amount of heartwood extractives linked to termite resistance in *Eucalyptus* spp.

*E. bosistoana* can grow in warm temperate climates up to 30-40 m in height, and usually has excellent stiffness, high density and hardness (Bootle, 2005; Poynton, 1979). In addition, *E. bosistoana* is classified as class 1 durable (Australian Standard, 2003), having a life expectancy of more than 25 years in-ground. The New Zealand Dryland Forests Initiative (NZDFI) identified *E. bosistoana* as the main species to establish an alternative durable wood plantation in New Zealand to meet the insufficient supply of ground durable timber (Li and Altaner, 2017). There are very few studies on the performance variability of *E. bosistoana*, particularly of its durability as a plantation species (Apiolaza et al., 2011; Davies et al., 2017).

In this study, the influence of genetic and environmental effects on the variability of properties associated to durability of *E. bosistoana* were examined, using samples taken from two first-generation progeny trials. Heartwood diameter (HWD), sapwood diameter (SWD)

sapwood area (SWA), heartwood extractive content (EC) and growth traits were examined. Near infrared reflectance (NIR) spectroscopy was evaluated as a rapid and cost-effective method to screen samples for EC.

## 6.2 Materials and methods

### 6.2.1 Materials

The New Zealand Drylands Forest Initiative (NZDFI) planted two *E. bosistoana* progeny trials in 2010. The trials represented 41 open-pollinated families, established in Martin (Canterbury) and Craven's Road (Marlborough) on New Zealand's South Island. Table 6-1 shows a summary of the characteristics of the two sites.

All trees were planted in an alpha lattice incomplete-block, single-tree-plot design. Incomplete blocks were 12 m × 10.8 m, with 30 trees per plot using a 2.4 m × 1.8 m spacing. Each tree represented one family, and no family was repeated within a block. Trials were thinned and pruned in Craven's Road at age six. There was one family in the Martin and five families in Craven's Road with no surviving trees.

**Table 6-1 Main site characteristics of *E. bosistoana* family trials**

Site	Longitude	Latitude	Families sampled	Trees sampled
Martin	172° 39'	43° 11'	40	1115
Craven's Road	173° 56'	41° 26'	35	650

### 6.2.2 Measurements

Stem diameter at breast height (DBH) was measured in 2017. Samples were collected in May and June 2017, coring all living trees in the trials with a battery-powered, 14 mm inner-diameter increment corer. Bark-to-bark stem cores through the pith were taken ~50 cm above

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the ground. In total, 1115 trees were cored at Martin and 650 trees at Craven's Road. Each core was collected into a cooling box and measured for core length under bark and HWD with a ruler in the green state on the day of collection. The heartwood was highlighted on the cores using a pH indicator (methyl orange). Heartwood changed colour to pink, while the colour of sapwood remained unchanged. SWD was calculated as the difference between the core length and HWD. SWA was calculated assuming circular a cross-section.

### 6.2.3 NIR spectroscopy and model calibration

Measurements of EC and decay resistance in laboratory test or field trials are time- and cost-consuming. Near infrared spectroscopy was investigated for rapidly and effectively measuring the EC in thousands of tree cores. Each core was placed in an air controlled room for a month (25 °C, 60% RH) to obtain a stable moisture content (~9%).

NIR spectra were collected from each core sample at the radial-tangential plane after it had been sanded using a P 100 sandpaper with a fibre optics probe (Model N-500, Bruker Optics, Germany) every 5 mm along the heartwood. Spectra were acquired from 9,000 to 4,000 cm<sup>-1</sup> at 4 cm<sup>-1</sup> intervals. Thirty-two scans were averaged for each spectrum. EC was predicted for each spectra using a PLS calibration developed by Li and Altaner (2018). The mean EC in heartwood was calculated as an area-weighted average.

### 6.2.4 Statistical analysis

Estimates of genetic parameters were obtained by fitting bivariate linear mixed models with restricted maximum likelihood (REML) analysis. Considering a single-trait observation  $y_i$  for a tree, it can be represented by the following model equation:

$$y_i = \mathbf{x}_i \mathbf{m} + b_i + f_i + e_i \quad (1)$$

where  $\mathbf{x}_i$  is a vector linking the fixed effects  $\mathbf{m}$  to the observation, and  $b_i$ ,  $f_i$  and  $e_i$  are the random block, family and residual effects. Expanding the notation to a bivariate case, for

each individual we have a vector of two observations  $\mathbf{y}_i$  (phenotypes for trait 1 and 2), and random vectors  $\mathbf{b}_i$ ,  $\mathbf{f}_i$  and  $\mathbf{e}_i$  for blocks, families and residuals. Stacking those vectors for all trees produces the model equation:

$$\mathbf{y} = \mathbf{X}\mathbf{m} + \mathbf{Z}_1\mathbf{b} + \mathbf{Z}_2\mathbf{f} + \mathbf{e} \quad (2)$$

where  $\mathbf{y}$  is a vector of phenotypic observations,  $\mathbf{m}$  is the vector of fixed effects (overall mean),  $\mathbf{b}$ ,  $\mathbf{f}$  and  $\mathbf{e}$  are vectors of bivariate random effects for block, family and residual effects.  $\mathbf{X}$ ,  $\mathbf{Z}_1$  and  $\mathbf{Z}_2$  are incidence matrices linking observations to the appropriate effects. The vector of expected values (E) and dispersion matrices (Var) were defined as:

$$E[\mathbf{y}] = \mathbf{X}\mathbf{m}$$

$$Var[\mathbf{b}] = \mathbf{Z}_{2\otimes}\mathbf{B}_0$$

$$Var[\mathbf{f}] = \mathbf{Z}_{2\otimes}\mathbf{F}_0$$

$$Var[\mathbf{e}] = \mathbf{Z}_{\oplus}\mathbf{R}_0$$

where  $\otimes$  and  $\oplus$  are the direct product and direct sum operations respectively, and

$$\mathbf{B}_0 = \begin{bmatrix} \sigma_{b1}^2 & \sigma_{b1b2} \\ \sigma_{b1b2} & \sigma_{b2}^2 \end{bmatrix}$$

$$\mathbf{F}_0 = \begin{bmatrix} \sigma_{f1}^2 & \sigma_{f1f2} \\ \sigma_{f1f2} & \sigma_{f2}^2 \end{bmatrix}$$

$$\mathbf{R}_0 = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e1e2} \\ \sigma_{e1e2} & \sigma_{e2}^2 \end{bmatrix}$$

where  $\sigma_{b_i}^2$ ,  $\sigma_{f_i}^2$  and  $\sigma_{e_i}^2$  represent the block, family and residual variances for trait  $i$ , and  $\sigma_{b_ib_j}^2$ ,  $\sigma_{f_if_j}^2$  and  $\sigma_{e_ie_j}^2$  are the block, family and residual covariances between traits  $i$  and trait  $j$ . The

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block and residual covariances were 0 when analysing the same trait across sites, as there was no information to estimate them.

The narrow sense heritability ( $h^2$ ) of trait  $i$  was estimated by using variance components from model

$$h_i^2 = \frac{2.5\sigma_{f_i}^2}{\sigma_{f_i}^2 + \sigma_{b_i}^2 + \sigma_{e_i}^2} \quad (3)$$

where  $\sigma_{f_i}^2$ ,  $\sigma_{b_i}^2$  and  $\sigma_{e_i}^2$  are respectively the family, block and residual variance for trait  $i$ . The genetic correlations ( $r_{g_{ij}}$ ) and phenotypic correlation ( $r_{p_{ij}}$ ) between trait  $i$  and trait  $j$  were calculated as

$$r_{g_{ij}} = \frac{\sigma_{f_{ij}}}{\sqrt{\sigma_{f_i}^2 + \sigma_{f_j}^2}} \quad (4)$$

$$r_{p_{ij}} = \frac{\sigma_{f_{ij}} + \sigma_{e_{ij}}}{\sqrt{(\sigma_{f_i}^2 + \sigma_{e_i}^2)(\sigma_{f_j}^2 + \sigma_{e_j}^2)}} \quad (5)$$

where  $\sigma_{f_{ij}}$  is the estimated family covariance between trait  $i$  and trait  $j$ ,  $\sigma_{f_i}^2$  is the estimated family variance for trait  $i$ , and  $\sigma_{f_j}^2$  is the estimated family variance for trait  $j$ . The realized genetic gain ( $\Delta G_R$ ) was computed by subtracting the mean breeding values of selected top ratio wood traits from the total mean of the wood trait. The difference was subsequently calculated in each site.

All models were fit using the asreml-R package (Gilmour et al. 2009) in the R statistical system (R Core Team, 2017).

### 6.3 Results and discussion

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### 6.3.1 Differences between family and site in growth and heartwood properties

Table 6-2 presents summary statistics for the six traits at Martin and Craven's Road. In both sites, the coefficients of phenotypic variation (CV) were highest for HWD followed by SWA, EC, DBH and SWD. There were site-level differences for the wood traits, with Craven's Road showing the higher mean for DBH, SWD and HWD but the lower mean for EC. In detail, DBH and HWD for trees at Craven's Road were with 101.8 mm and 42.1 mm significantly larger than those at Martin where DBH averaged 64.18 mm and HWD 31.7 mm. The mean annual increment in DBH was 14.5 mm/year at Craven's Road and 9.2 mm/year at Martin. The mean SWA were 84.5 and 55.6 cm<sup>2</sup> in Craven's Road and Martin, respectively. However, these larger trees at Craven's Road had a lower mean EC (7.7%) than those at Martin (9.6%). The EC means were lower than the 12% methanol soluble extractives reported for *E. cladocalyx* at 8 years of age (Bush et al. (2011)). However, apart from difference between species, older trees are known to produce more extractives than younger trees (Rudman, 1964) and methanol extracts slightly different compound mixtures than ethanol which was used in this study.

The data showed that trees at Craven's Road produced more heartwood and more sapwood. As trees at Craven's Road produced less heartwood extractives during growth than at Martin suggested that fast-growing trees usually produce less EC.

Heritability estimates used a coefficient of relationship of  $1/2.5$  in our study, which differed from (Apiolaza et al., 2011), who used  $1/4$  for half-sibling *E. bosistoana* families. The genetic structure of eucalypt populations is known to be complex due to inbreeding and hybridisation (Elliott and Byrne, 2003; Hunde et al., 2007; McDonald et al., 2003). However, there is no published information on both the reproductive biology and population structure of *E. bosistoana* to make informed assumptions. Lower relatedness coefficients pushed heritability

estimates outside the parameter space ( $h^2 > 1$ ), suggesting deviations from the assembling of half-siblings and the presence of inbreeding effects.

Moderate individual heritability estimates were found for most wood traits (Table 6-2). The highest heritability estimates were for DBH at Martin ( $h^2 = 1.11$ ). The estimated heritability for HWD in both sites were similar with 0.66 and 0.71. These values were higher than the findings of Bush et al. (2011), who estimated heritability of 0.3 to 0.38 for heartwood proportion in *E. cladocalyx* and Santos et al. (2004) who presented a heritability of 0.39 for the sapwood/heartwood ratio in *E. grandis*. EC was less heritable ( $h^2 = 0.16$  in Martin and 0.25 in Craven's Road), which was lower in one of the sites than the results reported by Wu et al. (2017) for EC of 0.26 for *E. grandis* and *E. urophylla* hybrids and by Bush et al. (2011) for EC of 0.25 of *E. cladocalyx*. The estimates of heritability for DBH at age 7 were 0.69 and 1.11 for the two sites. These were higher than the reported  $h^2$  of 0.13 estimated at age 7 for *E. globulus* (Costa e Silva et al., 2009). The high and moderate heritabilities for HWD and EC (Table 6-2), respectively, together with the substantial variability, suggested potential for improving heartwood quantity and quality via selection for *E. bosistoana*.

**Table 6-2 Descriptive statistics of growth and heartwood traits of 7-year-old *E. bosistoana* grown in Canterbury and Marlborough, New Zealand. DBH: diameter at breast height, EC: heartwood extractive content, HWD: Heartwood diameter, SWA: sapwood area, SWD: sapwood diameter.**

Site	Traits	Minimum	Maximum	Mean	CV (%)	SD	$h^2$
Martin	HWD (mm)	3	82	31.73	51	16.01	0.66 (0.12)
	SWD (mm)	20	144	60.38	26	15.46	0.66 (0.10)
	DBH (mm)	30	140	64.18	32	20.45	1.11 (0.12)
	EC (%)	1.83	22.38	9.63	42	4.09	0.16 (0.05)
	SWA (cm <sup>2</sup> )	11.94	149.74	55.55	44	24.66	1.24 (0.12)

	HWD (mm)	3	175	42.1	61	25.85	0.71 (0.11)
Craven's Road	SWD (mm)	18	135	70.28	25	17.74	0.53 (0.10)
	DBH (mm)	33	230	101.8	33	33.56	0.69 (0.11)
	EC (%)	0.77	18.02	7.67	46	3.54	0.25 (0.08)
	SWA (cm <sup>2</sup> )	14.17	263.89	84.51	51	43.07	0.91 (0.13)

### 6.3.2 Phenotypic and genetic correlations between traits

Table 6-3 shows the estimated genetic and phenotypic correlations between different traits at Martin and Craven's Road. Genetic correlations were high between HWD and DBH ( $r_g = 0.89$  in Martin and  $r_g = 0.98$  in Craven's Road). Martin had the smaller DBH and smaller heartwood diameter distribution, which suggested that tree growth constrained heartwood diameter growth. In summary, big trees yield larger heartwood diameter than small trees. Similar positive correlations have been reported among DBH and heartwood diameter in *P. pinaster* (Pinto et al., 2004), *P. radiata* (Wilkes, 1991), and *E. globulus* (Miranda et al., 2009).

No genetic correlation (0.13) and weak phenotypic correlation (0.37) was found between HWD and EC at Martin, while at Craven's Road significant negative genetic correlations between EC and other traits, including HWD (-0.86), SWD (-0.89) and DBH (-0.86) were detected. In 2015 this site was pruned and small trees thinned, which may have contributed to the differences between sites. Negative correlations between growth traits and EC were also reported by Wu et al. (2017) for *Eucalyptus* hybrid clones. Therefore growing large-diameter trees on fast-growing sites will not necessarily result in the most valuable plantations if durable wood is the target product.



**Table 6-3 Phenotypic (above diagonal) and genetic correlations (below diagonal) between traits with standard error between parentheses. DBH: diameter at breast height, EC: heartwood extractive content, HWD: Heartwood diameter, SWA: sapwood area, SWD: sapwood diameter.**

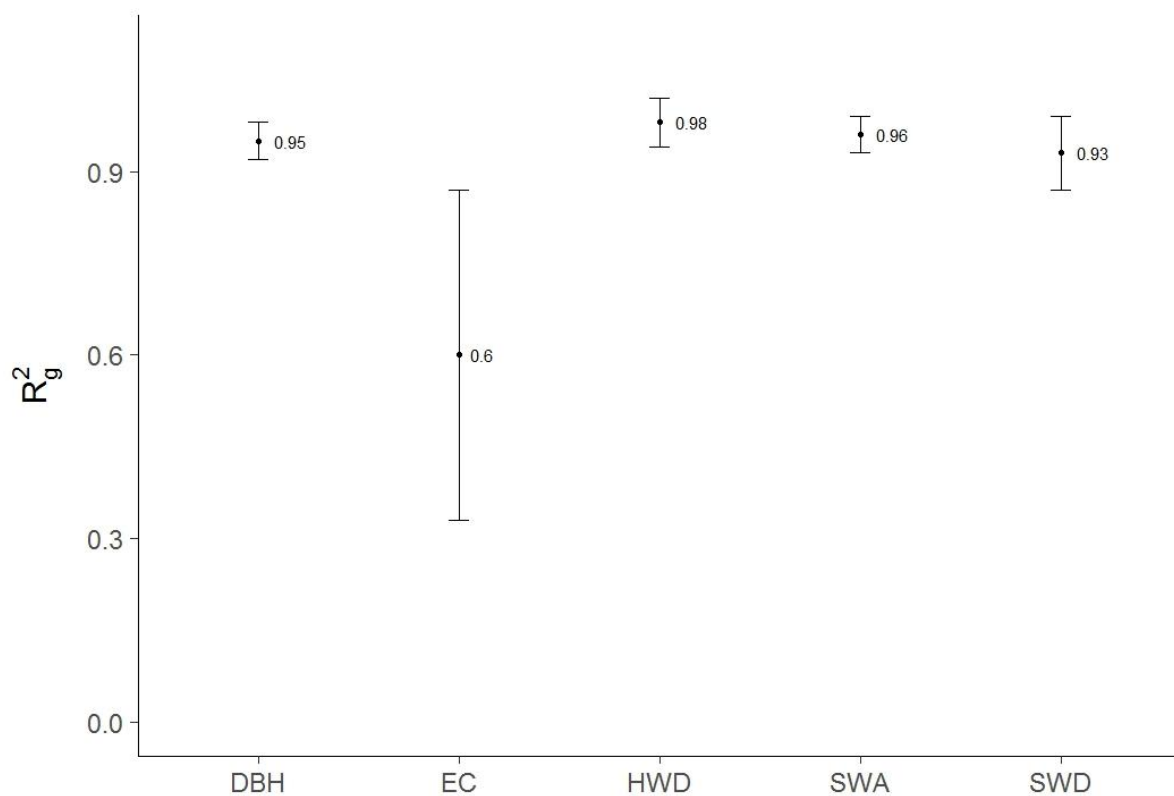
	Traits	HWD	SWD	DBH	EC	SWA
Martin	HWD		-0.08 (0.06)	0.59 (0.04)	0.37 (0.04)	0.60 (0.04)
	SWD	0.47 (0.16)		0.43 (0.05)	-0.22 (0.04)	0.76 (0.02)
	DBH	0.89 (0.04)	0.76 (0.08)		0.15(0.04)	0.81 (0.02)
	EC	0.13 (0.23)	-0.52 (0.18)	-0.18 (0.13)		0.02 (0.05)
	SWA	0.85 (0.06)	0.89 (0.04)	0.99 (0.01)	-0.33 (0.21)	
Craven's Road	HWD		0.17 (0.08)	0.78 (0.03)	0.03 (0.07)	0.67 (0.04)
	SWD	0.97 (0.07)		0.58 (0.04)	-0.29 (0.06)	0.87 (0.01)
	DBH	0.98 (0.01)	0.88 (0.05)		-0.14 (0.06)	0.90 (0.01)
	EC	-0.86 (0.18)	-0.89 (0.11)	-0.86 (0.14)		-0.22 (0.06)
	SWA	0.98 (0.03)	0.99 (0.01)	0.98 (0.01)	-0.85 (0.13)	

### 6.3.3 Site influence on wood traits

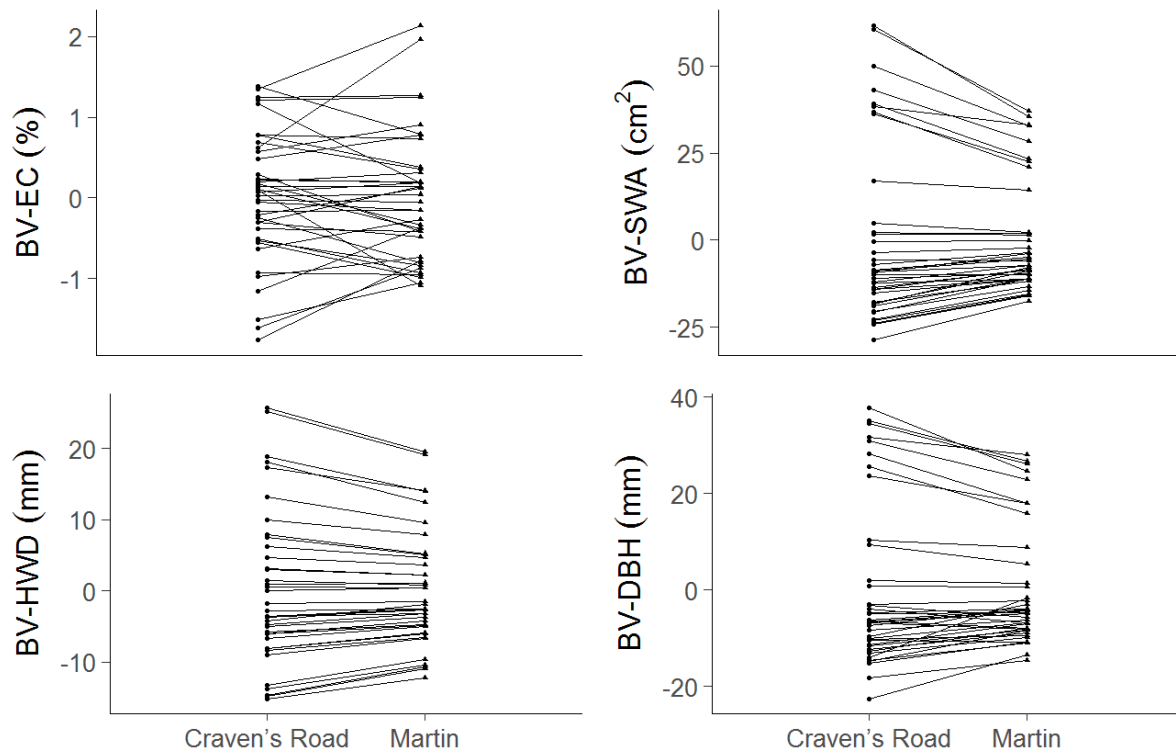
Genotype by Environment ( $G \times E$ ) interaction is a common preoccupation in breeding for growth traits (Alía et al., 1997; Costa e Silva et al., 2006; Ivković et al., 2015). In multivariate genetic evaluation, genetic correlations between the same traits in different sites can be interpreted as a measure of  $G \times E$  interaction. Consistent rankings across sites (low  $G \times E$  interaction) are reflected as high genetic correlations.

Figure 6-1 shows the genetic correlations across sites (and their standard errors) for all traits, while Figure 6-2 displays the across-site stability for the predicted family values. Rankings were consistent across the two sites for HWD, SWA, SWD, DBH indicated by the high

genetic correlations of 0.98, 0.96, 0.93 and 0.95, respectively. Even the lowest genetic correlation (0.6 for EC) suggested only moderate changes in the rankings between sites. Nevertheless, it is important to remember that this is a sample of only two sites, and there is evidence of more variable performance between sites for *E. bosistoana* in terms of tree height (e.g. Apiolaza et al. 2011).



**Figure 6-1 Genetic correlations ( $R_g^2$ ) of growth and heartwood traits for *E. bosistoana* at age 7 between two sites. Bars denoted standard errors. DBH: diameter at breast height, EC: heartwood extractive content, HWD: Heartwood diameter, SWA: sapwood area, SWD: sapwood diameter.**



**Figure 6-2** Changes of family rankings across sites for heartwood diameter (HWD), DBH, extractives content (EC) and sapwood area (SWA) for *E. bosistoana* at age seven between two sites. Family values are expressed as deviation from the site mean. BV: Breeding values, DBH: diameter at breast height, EC: heartwood extractive content, HWD: Heartwood diameter, SWA: sapwood area.

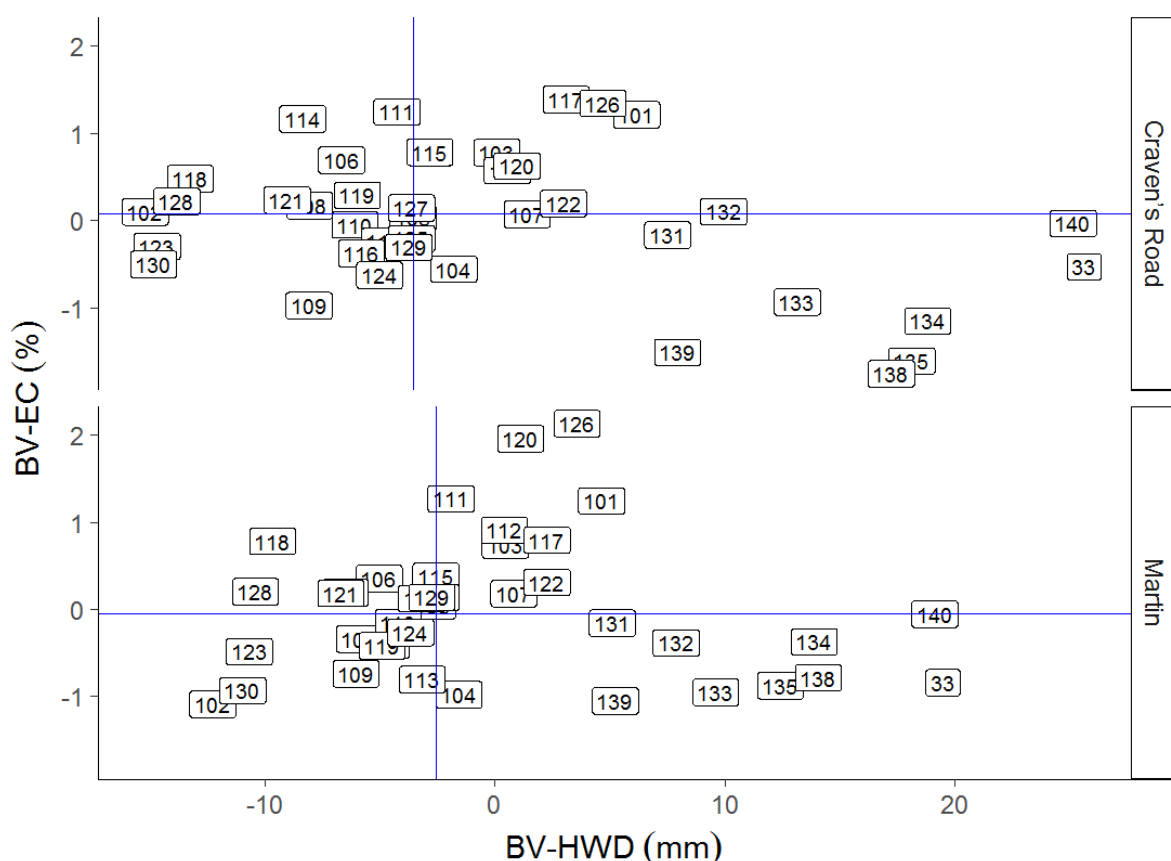


Figure 6-3 Relationship between extractives content (EC) and heartwood diameter (HWD) breeding values of *E. bosistoana* families at age 7 in two sites.

#### 6.3.4 Family selection

Quantity and quality of heartwood are the main timber traits in the NZDFI breeding programme. Families that have both large HWD and high EC should be preferred for breeding. Figure 6-3 presents the mean of HWD and EC relationship in both sites. It is clear that some families have large HWD on both sides, including families 133, 134, 135, 138, 139, but have lower EC. These families should not be selected as low EC is likely to result in low natural durability. Similarly families, which produced high EC but small HWD in both sites, for example 118, 121 or 128, should not be selected. In contrast, despite the negative correlation between EC and HWD (Table 6-3), some families (including 101, 112, 117, 120, 126) produced above average HWD and EC and should be selected. Families, which

performed above average for both, HWD and EC, in only one of the sites (e.g. 132 and 115 at Craven's Road and 107 and 111 at Martin) have potential when matched to site conditions.

### 6.3.5 Realized genetic gains

The realised genetic gains when selecting the top 10%, 20% and 30% of the families for individual traits are presented in Table 6-4. Genetic gains increased with stronger selection rates for all traits. Estimated genetic gains for DBH in both sites ranged from 17.8 to 34.7 mm. This compared to gains of 11.6 mm to 24 mm per year reported for *E. globulus* when selecting the top 0.6% of individuals (Jarvis et al., 1995). However, since for *E. bosistoana* the target trait is heartwood, selection for DBH is not appropriate. Instead, selection should consider HWD, which gave estimated gains from 21.9 mm to 14 mm for Craven's Road and from 16.7 mm to 10.5 mm for Martin. Larger genetic gain for HWD, SWD, DBH and SWA can be expected for sites comparable to Craven's Road. Growing conditions similar to those at Martins only slightly favoured realised genetic gain for EC.

**Table 6-4 Realised genetic gains of growth and heartwood traits at age 7 for *E. bosistoana*. DBH: diameter at breast height, EC: heartwood extractive content, HWD: Heartwood diameter, SWA: sapwood area, SWD: sapwood diameter.**

Top selection ratio	Martin			Craven's Road		
	10%	20%	30%	10%	20%	30%
HWD (mm)	16.65	13.79	10.46	21.94	18.30	14.04
SWD (mm)	15.34	11.47	8.8	19.29	14.54	11.02
DBH (mm)	26.34	23.43	17.75	34.74	31.94	24.42
EC (%)	1.65	1.30	0.99	1.30	1.13	0.94
SWA (cm <sup>2</sup> )	34.55	30.33	22.90	53.68	46.99	35.37

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## 6.4 Conclusions

We observed high variation and relatively high heritabilities of HWD ( $h^2 = 0.66-0.71$ ) and DBH ( $h^2 = 0.69-1.11$ ) for 7 year-old *E. bosistoana* at all sites. It was possible to identify families with superior HWD and EC despite their negative genetic correlation.

There was no practically significant  $G \times E$  interaction for growth traits, while there was a small level of  $G \times E$  interaction for EC. We observed both phenotypic and genetic variation for HWD and EC between the families, indicating that there is potential to improve heartwood quantity and quality in *E. bosistoana* by selection to ensure trees with abundant heartwood of good quality.

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## 6.5 References

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